On the relevance of thrombomodulin variants in atypical hemolytic uremic syndrome

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Atypical hemolytic uremic syndrome (aHUS) is a potentially lethal medical condition often resulting in chronic kidney disease.[1] Genetic testing is critical in clinical care because pathogenic variants in complement genes account for 50% to 60% of cases of aHUS,[1,2] and their identification reinforces diagnosis and assists clinical decisions, such as treatment with anti-complement drugs.[1,3] One gene of interest is *THBD*, which encodes thrombomodulin (TM), an anticoagulant endothelial membrane glycoprotein that also regulates complement by binding to factor H and accelerating the inactivation of C3b.[4,5] Several years ago, it was reported that *THBD* variants altering the complement-regulatory activities of TM predispose to aHUS.[4] However, studies confirming the *THBD*-aHUS association and determining whether the complement-regulatory activities of TM are significant in aHUS are lacking. This study aims to fill this knowledge gap by investigating *THBD* variants in the Spanish aHUS/C3-glomerulopathy registry. Herein, we report the genetic and clinical retrospective study of 27 patients carrying *THBD* variants, with a critical view of the *THBD*-aHUS association that questions the relevance of TM variants to aHUS development.

METHODS

The aHUSC3G registry is a repository of clinical and research data focused on aHUS and C3G Spanish patients, established to facilitate analysis by researchers. The study population consisted of 27 aHUS patients with identified THBD genetic variants from the registry, and their outcomes were assessed in terms of hematologic and renal responses. Genetic analyses were conducted using next-generation sequencing, and a gene-based collapsing test was performed to compare the prevalence of variants in different genes between the registry and the gnomAD database. Statistical analysis was carried out using Stata version 14 (Supplementary Methods).

RESULTS

The study includes all carriers of *THBD* variants with an allele frequency <1% in the European non-Finnish population of

the Genome Aggregation Database (Supplementary Methods). Twenty-seven patients with aHUS from our registry, representing 2.22% from 1216 patients, carry 1 of 14 different *THBD* variants in heterozygosity (Table 1); 20 carry isolated *THBD* variants, whereas 7 have *THBD* variants concurrent with a well-characterized pathogenic variant in the *C3*, *CFI*, *CFH*, *CFB*, or *MCP* genes. *THBD* variants known to alter the function of TM were clasified as functional variants. Otherwise, we classified *THBD* variants as "potentially functional" or "likely benign" based on their combined annotation-dependent depletion scores (Table 1; Supplementary Tables S1 and S2, and Supplementary Methods). Nineteen patients carry *THBD* variants classified as functional or potentially functional, 12 of them isolated and 7 in concurrence with a complement pathogenic variant (Supplementary Tables S1 and S2).

The criteria used by Delvaeye *et al.*[4] to include *THBD* variants in their study are similar to those applied here of including variants with allele frequency <1% in the European non-Finnish population (Supplementary Methods). However, the relevance of some of these *THBD* variants in aHUS, despite their reported capacity to modify the complement-regulatory function of TM, is questionable. This is because the allele frequency in some populations is too high to be considered a genuine genetic risk factor for an ultrarare disease like aHUS (Figure 1a). Notably, the frequency of carriers with *THBD* variants, having an allele frequency of <1%, is 1.95% in the European non-Finnish population of Genome Aggregation Database (1216 of 64,603; Supplementary Table S3). These numbers are similar to the 1.84% (6 of 326; Supplementary Table S4) observed in our cohort of patients with C3-glomerulopathy (Genome Aggregation Database vs. C3-glomerulopathy, $P = 0.887$) and both, 1.95% and 1.84%, are close to the 2.22% of carriers identified in our cohort with aHUS (Genome Aggregation Database vs. aHUS, $P = 0.498$; C3-glomerulopathy vs. aHUS, $P = 0.674$). To assess whether our cohort with aHUS is enriched in rare/functional *THBD* variants, we performed a gene-based collapsing test comparing the numbers of carriers of *THBD* variants between our cohort with aHUS and the European non-Finnish population (Figure 1b and Supplementary Methods). As a control, we included in these analyses 3 complement genes in which loss-of-function pathogenic variants have been unquestionably associated with aHUS. Our data, showing no enrichment, are consistent with previous analyses from different aHUS reference centers. [6,7]

Of the 14 patients with medical records available, 12 carry isolated *THBD* variants, and 2 have the *THBD* variants concurrent with a pathogenic variant in *C3* (Table 1). Nine of these 14 patients carry functional *THBD* variants included in the original report by Delvaeye *et al.*,[4] and 3 carry *THBD* variants reported associated with aHUS.[7] None of the 14 patients had a family history of thrombotic microangiopathy.

Among the 12 patients with isolated *THBD* variants, 6 had a personal history of chronic kidney disease of different cause than aHUS (Table 1), and 5 of them had the thrombotic microangiopathy event in a transplanted kidney (Supplementary Table S5). Five of the 12 patients required renal replacement therapy: 4 received a kidney transplant, and a fifth remained on hemodialysis (Table 1).

In 9 of the 12 patients, the HUS event was associated with a kidney transplant (n = 5), drugs (n = 3), infection (n = 2), ischemia-reperfusion damage (n = 1), and postpartum effects (n = 1; Table 1). At presentation, 9 patients had high blood pressure; and in 4 who underwent fundus examination, 3 had grade III/IV retinopathy. Six patients manifested digestive symptoms, 2 had neurologic involvement, and 2 had cardiac involvement. In 7 of the 12 cases, thrombotic microangiopathy was confirmed by kidney biopsy (Table 1). Four patients were treated with eculizumab without conclusive results (Table 1); and in all patients, eculizumab was suspended without recurrences (Table 1). Notably, all 8 untreated patients, managed only by removing the putative triggers, had a hematological response, with 4 of 8 (50%) having a full kidney response and 3 of 8 (37.5%) having a partial kidney response (Table 1). There were no cases of recurrence among the 12 patients with isolated *THBD* variants, although there were a total of 4 kidney transplants after the thrombotic microangiopathy event without prophylactic eculizumab treatment (Table 1 and Supplementary Tables S5 and S6).

Both patients with the concurrent *C3* variant had severe acute kidney failure requiring immediate hemodialysis. Hemoglobin decrease was also more striking in these patients (minimum, 6.3 g/dl) compared with patients with isolated *THBD* variants (Supplementary Table S6). One of these patients was treated with eculizumab 3 days after presentation, with excellent hematological and kidney response. In the untreated patient, the hematological alterations improved after the disappearance of the trigger (cocaine), but without achieving renal recovery (Supplementary Table S6).

DISCUSSION

The absence of studies replicating the original report associating the development of aHUS with *THBD* variants[4] generates uncertainty regarding the management of patients with suspected complement-mediated aHUS, but only isolated *THBD* variants have been identified through genetic testing.[2] We identified 27 patients carrying *THBD* variants among 1216 patients with aHUS, all of them with a comprehensive genetic study, including screening of all aHUS genetic risk factors and the search for anti–factor H autoantibodies (Supplementary Methods), and we were able to retrieve the complete clinical data for 14 of them. More important, 12 patients presented isolated *THBD* variants, which allowed us to evaluate the contribution of the *THBD* variants to the development of aHUS in the absence of any other genetic or acquired aHUS risk factor.

Our study has identified important weaknesses in the proposed association between *THBD* variants and the development of aHUS, as outlined in the original *New England Journal of Medicine* report.[4] Specifically, because of a lack of

Table 1 | Clinical and genetic characteristics of patients with *THBD* variants

	Baseline data						TMA onset		Treatment and response			Long-term evolution		Genetics		
ID[a]	Age, yr	Sex	Pregnancies (abortions)	CKD	RRT (before TMA)	sCr, mg/dl	Secondary causes	TMA-positive kidney biopsy[b]	Eculizumab (days from onset)	Treatment ECU, d	Recovery	Relapse follow-up, yr	RRT[c]	*THBD* variant	Classification	Additional complement variants
1	52	M	M	NAE	D and KT	2.1	N	Y	N	N	Hematol. RRT	N (0.9)	D	Ala43Thr	Functional	N
2	60	M	M	FSGS	N	2.8	N	Y	N	N	Hematol. kidney partial	N (2.6)	N	Glu569Lys	Likely benign	N
3	26	M	M	Nephronophthisis	D, KT (lost), and D again	D	N	ND	Y (1)	133	Hematol. previous HD	N (0.4)	Previous HD	Asp486Tyr	Functional	N
4	53	F	1 (0)	N	N	0.8	N	Y	N	N	Hematol. kidney full	N (2.5)	N	Pro501Leu	Functional	N
5	56	F	2 (1)	N	N	0.8	N	Y	Y (2)	323	Hematol. kidney partial	N (1.0)	N	Asp486Tyr	Functional	N
6	4 mo	F	0	N	N	0.9	GI infection (Shiga toxin–negative, no DIC)	ND	N	N	Hematol. kidney full	N (13.8)	N	Tyr39Phe	Likely benign	N
7	18 mo	M	M	N	N	0.8	GI infection (Shiga toxin–negative, no DIC)	ND	N	N	Hematol. kidney full	N (23.1)	D; KT (18.4)	Asp418Thrfs*88	Functional	N
8	41	F	2 (1)	Kidney vascular disease (RAS)	D and KT (2)	4.0	IRI	ND	Y (94)	11	Hematol. RRT	N (5.9)	D; KT (4.4)	Arg403Lys	Likely benign	N
9	24	F	0	CNI	D and KT	D	CNI	ND	N	N	Hematol. kidney partial	N (6.4)	D; KT (3.3)	Ala43Thr	Functional	N
10	50	F	0	N	N	0.6	Gemcitabine	Y	Y (22)	64	Hematol. kidney partial	N (0.3)	N	Pro228Leu	Likely benign	N
11	13	M	M	AS	KT	0.8	Infection, drugs	Y	N	N	Hematol. kidney full	N (0.7)	N	Arg403Lys	Likely benign	N
12	23	F	0	N	N	NA	Pregnancy, severe bleeding, but no DIC	Y	N	N	Hematol. kidney partial	N (23.1)	D, KT (23.0)	Glu293Asp	Likely benign	N
13	51	F	4 (0)	N	N	1.6	Cocaine	Y	N	N	Hematol. RRT	N (2.1)	D	Pro501Leu	Functional	C3 (Lys65Gln)
14	44	F	3 (0)	N	N	0.9	Viral infection (flu-like symptoms, no DIC)	ND	Y (3)	Ongoing	Hematol. kidney full	N (1.9)	N	Ala43Thr	Functional	C3 (Lys65Gln)

AS, Alport syndrome; CKD, chronic kidney disease (cause); CNI, calcineurin inhibitor; D, dialysis; DIC, disseminated intravascular coagulation; ECU, eculizumab; F, female; FSGS, focal segmental glomerulosclerosis; GI, gastrointestinal; hematol., hematological; HD, hemodialysis; ID, patient identifier; IRI, ischemia/reperfusion; KT, kidney transplant; M, male; N, no; NA, not available; NAE, nephroangiesclerosis; ND, not done; RAS, renal artery stenosis; RRT, renal replacement therapy; sCr, serum creatinine; TMA, thrombotic microangiopathy; Y, yes.

[a]Patients 15 to 27, without clinical data, are depicted in Supplementary Table S1.

[b]Biopsy results did not show lesions suggesting other diagnosis.

[c]In parenthesis, years from onset to kidney transplant.

a

gnomAD (n = 141,456)	ENF (n = 64,603)	Africans (n = 12,487)	Latin (n = 17,720)
A473V (19%)	A473V (19%)	A473V (5%)	A473V (18,6%)
D486Y (0.2%)	**A43T (0.36%)**	G502R (2%)	**D486Y (5%)**
G502R (0.16%)	**P501L (0.29%)**	G14S (1.7%)	R403K (0.5%)
A43T (0.1%)	P495S (0.11%)	P368S (0.12%)	**A43T (0.15%)**
P501L (0.05%)	P228L (0.05%)	V454A (0.12%)	**P501L (0.1%)**
G14S (0.02%)	**D486Y (0.02%)**	A236G (0.12%)	H58Y (0.07%)
R403K (0.019%)	P508L (0.01%)	**A43T (0.08%)**	G502R (0.05%)

b

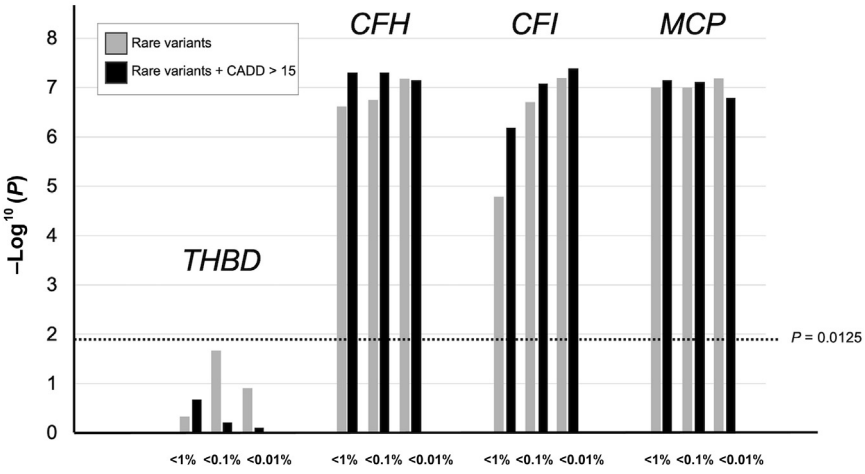

Figure 1 | Functional *THBD* variants are frequent in some ethnic groups and show no enrichment in atypical hemolytic uremic syndrome (aHUS) compared with the normal population. (**a**) Most frequent *THBD* variants in different populations included in Genome Aggregation Database (gnomAD). *THBD* functional variants that have been shown to alter the complement-regulatory function or thrombomodulin[4] are depicted in bold. (**b**) Gene-based collapsing test showing no enrichment of *THBD* variants in the Spanish cohort with aHUS. A χ^2 analysis was used to compare the number of carriers of variants in the *THBD*, *CFH*, *CFI*, and *MCP* (*CD46*) genes with allele frequencies of 1%, 0.1%, and 0.01% in gnomAD and in the cohort with aHUS, and the negative log base 10 of the significance *P* value for each of the comparisons was calculated (gray columns). A similar calculation was made for potentially functional variants (combined annotation-dependent depletion [CADD] scores >15; black columns). The dotted, horizontal line indicates the significance threshold ($P = 0.0125$) with a Bonferroni correction. As expected, a highly significant enrichment for both rare and rare + pathogenic variants is observed for the *CFH*, *CFI*, and *MCP* (*CD46*) genes, whereas rare and rare + functional *THBD* variants are not enriched. ENF, European non-Finnish.

information on allelic frequencies at the time, the authors overlooked that certain *THBD* variants are highly prevalent in other ethnic groups. This lack of information also hampers the authors to test whether their cohort with aHUS had an enrichment of rare/functional *THBD* variants compared with normal populations.

Clinical data show that, in our cohort, none of the patients carrying isolated *THBD* variants had a recurrence of the disease throughout the entire follow-up, including 4 patients who received a kidney graft without preemptive eculizumab, and most patients carrying isolated *THBD* variants had ≥1 cause associated with secondary HUS and tended to recover spontaneously with the removal of the putative underlying cause. Overall, our study illustrates that patients carrying *THBD* variants do not exhibit the prototypical clinical profiles of patients with complement-mediated aHUS and resemble those with secondary HUS.[3,8,9] This information is crucial for their therapeutic management.

In conclusion, we show that the *THBD* variants found in our cohort with aHUS merely reflect their prevalence in the normal population. Although our study has limitations because of the small number of patients and its retrospective nature, the data generated do not support a significant contribution of the *THBD* variants to the development of complement-mediated aHUS.

DISCLOSURE

Conception, design, data collection and analysis, as well as writing of the study were performed as an independent research initiative without support from pharmaceutical companies. SRdC, AH, MB, and NR have received honoraria from Alexion Pharmaceuticals for giving lectures and participating in advisory boards. None of these activities has had any influence on the results or interpretations in this article. The results presented in this article have not been published previously in whole or part. All the other authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Supplementary Methods.

Supplementary Table S1. Complement genetic and acquired risk factors in patients with atypical hemolytic uremic syndrome (aHUS) carrying *THBD* variants.

Supplementary Table S2. Prevalence and pathogenicity predictions for *THBD* variants found in the Spanish atypical hemolytic uremic syndrome (aHUS) cohort.

Supplementary Table S3. Carriers of *THBD* variants in the European non-Finnish (ENF) population from the Genome Aggregation Database (gnomAD; n = 64,603).

Supplementary Table S4. Carriers of *THBD* variants in the C3-glomerulopathy cohort (n = 326).

Supplementary Table S5. Hemolytic uremic syndrome in kidney grafts.

Supplementary Table S6. Hemolytic uremic syndrome in native kidneys.

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