Complement alternative pathway determines disease susceptibility and severity in antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis

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Activation of the alternative pathway (AP) of complement is involved in the pathogenesis of antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis (AAV), although the underlying molecular mechanisms are unclear. To gain insight into the role of the AP, common gene variants in *CFH/CFHR1-5, CFB, C3 and MCP*, and longitudinal determinations of plasma C3, C4, FH, FHR-1, FHR-2, FHR-5, FB, properdin and sC5b-9 levels were analyzed in a Spanish AAV cohort consisting of 102 patients; 54 with active AAV (active cohort) and 48 in remission not receiving immunosuppressants or dialysis therapy (remission cohort). The validation cohort consisted of 100 patients with ANCA-associated glomerulonephritis. Here, we demonstrated that common genetic variants in complement components of the AP are associated with disease susceptibility (*CFB32Q/W*) or severity of kidney damage in AAV (*CFH-H1, CFH1H2 and ΔCFHR3/1*). Plasma levels of complement components were significantly different between active and remission cohorts. In longitudinal observations, a high degree of AP activation at diagnosis was associated with worse disease outcome, while high basal FHR-1 levels and lower FH/FHR-1 ratios determined severe forms of kidney associated AAV. These genetic and plasmatic findings were confirmed in the validation cohort. Additionally, autoantibodies against FH and C3 convertase were identified in one and five active patients, respectively. Thus, our study identified key genetic and plasma components of the AP that determine disease susceptibility, prognosis, and severity in AAV. Our data also suggests that balance between FH and FHR-1 is critical and supports FHR-1 as a novel AP-specific therapeutic target in AAV.

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Lay Summary

The activation of the complement alternative pathway (AP) has been involved in the pathogenesis of antineutrophil cytoplasmic antibody–associated vasculitis (AAV), although the underlying molecular mechanisms are unclear. Here, we have identified common genetic variants in complement genes of the AP that are associated with protection to develop AAV (*CFB32Q/W*) or that are associated with increased severity of the disease (deletion of *CFHR1* and *CFHR3*, and *CFH-H1*). Consistent with these findings, circulating complement levels in active disease were altered compared with control and remission samples, and some of them have a prognostic value. Our data also suggest a crucial role of the balance between factor H (FH) and FH-related protein 1 in AAV. This study could explain the heterogeneity in prognosis and response to treatment observed in AAV.

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Antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis (AAV) is a systemic autoimmune disease that is characterized by necrotizing inflammation of predominantly small blood vessels and the presence of circulating ANCAs directed against myeloperoxidase or proteinase 3. The main histologic feature in the kidneys of patients with

AAV is "pauci-immune" necrotizing crescentic glomerulonephritis with absent or scarce Ig and complement deposits.[1,2]

The complement system was not, therefore, initially thought to be associated with the development of AAV. Convincing evidence from animal models and clinical observations indicates, however, that activation of the complement system, the alternative pathway (AP) in particular, is pivotal for the development of AAV.[3–5] C5a is a potent anaphylatoxin and chemoattractant, crucial in disease pathogenesis,[5] and blockade of C5a or C5a receptor (CD88) ameliorates anti-myeloperoxidase necrotizing and crescentic glomerulonephritis in mice.[6] Recently, a phase 3 clinical trial (ADVOCATE, NCT02994927) has shown that C5a receptor inhibition with avacopan, a small antagonist, was effective in replacing high-dose glucocorticoids in patients with AAV treated with rituximab or cyclophosphamide during induction treatment.[7]

The complement system is a major component of the innate immunity. On its activation through any of its 3 activation pathways (classical, lectin, or alternative), the complement system displays various effector functions, including induction of inflammation and cell damage. Unlike the classical and lectin pathways that need a stimulus to be triggered, the AP is always in "ready to fire" status; therefore, regulatory mechanisms are crucial to prevent uncontrolled complement activation. Factor H (FH) is the main regulator of the AP, both in fluid phase and on cellular surfaces. In certain circumstances, the regulatory activity of FH can be counteracted by FH-related proteins (FHRs; FHR1–5), other members of the FH protein family.[8] Unlike FH, the FHRs lack the complement regulatory domains of FH but share with FH similarities within the surface recognition domains. In contrast to FH, which inactivates surface-bound C3b and prevents further C3b generation and deposition (negative regulation), surface-bound FHRs promote complement activation through the AP.[9–12] Hence, the balance between FH and the FHRs may determine the outcome of AP activation/regulation and, as we previously reported for IgA nephropathy and C3 glomerulopathy, dysfunctions or alterations in the protein levels of either FH or FHRs may lead to the development of complement-mediated diseases.[13]

Although the involvement of complement in AAV pathogenesis is unquestionable, the exact molecular mechanisms leading to dysregulation of the AP are unclear. Here, we have characterized the circulating levels of various complement components and performed genetic association studies of common complement gene variants in AAV, and we investigated their contribution to disease susceptibility, severity, and prognosis in a Spanish cohort of AAV.

METHODS

Patients with AAV and control cohorts

A total of 202 adult patients with diagnosis of AAV with kidney involvement, belonging to 2 independent cohorts (discovery and validation cohorts) from 5 Spanish centers from 2016 and 2022, were included in the study. The discovery cohort consisted of 102 patients, and it was composed of 54 patients with a diagnosis of active AAV (active cohort) and 48 patients who were in remission and not receiving immunosuppressants or dialysis therapy (remission cohort). The validation cohort was composed of 100 patients with ANCA-associated glomerulonephritis. The validation cohort was mainly used to validate the genetic studies. Moreover, we gathered plasma samples from 25 age-matched controls. For the genetic studies, 389 healthy control samples of Caucasoid origin from the Spanish Registry of atypical hemolytic uremic syndrome and patients with C3 glomerulopathy (https://www.ahusc3g.es/) were used.

Patients from the discovery active cohort were longitudinally followed up for 12 months after diagnosis. Clinical visits were scheduled at diagnosis, month 1, month 2, and every 3 months thereafter. Plasma and urine samples were obtained before immunosuppressive treatment was started and at every clinical visit. In the discovery remission cohort, clinical data were recorded retrospectively, and plasma and urine samples were collected when the patient was in remission. Plasma samples of 10 patients of the validation cohort were included at the time of onset and were used to validate the plasma complement determinations observed in the discovery cohort.

Definitions and outcome

Kidney vasculitis was defined histologically by the presence of extracapillary proliferation associated with focal glomerular necrosis and/or small-vessel vasculitis, in the absence of significant glomerular immune deposits.[2] Renal biopsy was performed at disease onset. Patients with secondary vasculitis were excluded. Vasculitis disease activity was recorded using the Birmingham Vasculitis Activity Score (version 3).[14] Disease manifestations were scored when they were attributable to active vasculitis and occurred in the previous month (new onset or worsening).

Estimated glomerular filtration rate was measured using the Chronic Kidney Disease Epidemiology Collaboration creatinine formula.[15] Remission was defined by stable or decreasing serum creatinine concentration associated with significant improvement of hematuria as well as absence of extrarenal disease activity. Relapse was defined as the presence of active urine sediment and/or an increase in creatinine concentration by >30% attributable to active vasculitis after remission was achieved. The study was approved by the Research Ethics Committees of the Fundación Hospital Alcorcon, and all patients provided written informed consent.

Statistical analysis

Quantitative parameters were represented as mean and SD or median and the interquartile range. Categorical variables were expressed as frequencies or percentages. Some variables were depicted in logarithmic scale. Differences of quantitative parameters between groups were assessed using the unpaired *t* test or Mann-Whitney test, depending on the data distribution. Pearson correlations were performed to explore lineal relationships between FH, C3, factor B (FB), and properdin. Besides, to analyze the variation of the complement components in each patient, the Wilcoxon matched-pairs signed rank test was applied. $P < 0.05$ was considered significant. The comparison between allele frequencies of complement genetic variants were examined by χ^2 association test or Fisher exact test. Odd ratios and 95% confidence intervals were calculated. If allele frequencies were 0, the odds ratio could not be calculated and the 95% confidence interval was estimated using Cornfield correction. A Bonferroni correction for the 5 different loci studied was applied, and hence $P < 0.01$ was considered significant. Analyses were performed with GraphPad Prism 8 software and STATA software.

Complement genetic analyses (Supplementary Figure S1), quantification of plasma (Supplementary Table S1), and urine complement components, detection of autoantibodies, quantification of urinary soluble CD163 (usCD163), and creatinine in urine are detailed in the Supplementary Methods.

RESULTS

Baseline characteristics of patients with AVV

For the initial studies, 102 patients with a diagnosis of AAV and renal involvement were included in the study (global discovery cohort), of whom 54 patients were gathered with active disease (active cohort) and 48 patients were in remission (remission cohort). Of the patients with active AAV, 49 had newly diagnosed AAV, whereas 5 had relapsing AAV. In addition, a validation cohort consisting of 100 patients with AAV with renal involvement was used for replication studies. The clinical characteristics at the time of diagnosis of the discovery cohort (global and split active and remission patients) and of the validation cohort are listed in Table 1.

Analysis of common complement genetic variants

To investigate if genetic variations in complement genes influence the susceptibility to develop AAV, common variants in *CFH-CFHR1-5*, *C3*, *CFB*, and *MCP* that are known to modulate the activity of the alternative pathway were analysed in the discovery cohort (n = 99, as DNA was not available in 3 patients) and in a matched control population (n = 389). Comparison of the allele frequencies observed in AAV with those observed in controls shows that the *CFB32Q/W* alleles are significantly decreased (odds ratio, 0.585; 95% confidence interval, 0.394–0.867; $P = 0.008$) in patients with AAV, whereas no significant differences are observed in *CFH*, *C3*, *MCP*, or *ΔCFHR3/1* (Table 2). To validate these findings, the same genetic variants were analysed in the independent AAV validation cohort. As can be seen in Table 2, similar results were obtained, indicating that the *CFB32Q/W* alleles (odds ratio, 0.577; 95% confidence interval, 0.389–0.856; $P = 0.006$) are a protective factor for the development of AAV.

Complement plasma profile according to AAV status

Circulating levels of complement C3 and C4, FH, intact FB, properdin, and soluble C5b-9 (sC5b-9) were determined in plasma samples from both the active and remission patients from the discovery cohort and in healthy controls. In patients with active disease, the median concentrations of C3, FH, FB, and properdin were significantly decreased compared with patients in remission or controls (Figure 1; Supplementary Table S2). Conversely, sC5b-9 levels were significantly increased in active disease samples compared with remission or control samples. Moreover, a positive correlation was observed between FH and C3, FB, or properdin (Figure 2). Altogether, these data provide evidence of complement system activation through the AP and up to the terminal pathway during the active phase of the disease.

Table 1 | Baseline clinical and histopathologic data of active and remission AAV

Variable	Discovery cohort			Validation cohort
	Global (n = 102)	Active (n = 54)	Remission (n = 48)	Global (n = 100)
Age at diagnosis, yr	67 ± 15	70 ± 13	63 ± 17	66 ± 13
Male sex, n (%)	60 (59)	33 (60)	27 (57)	44 (44)
ANCA specificity, n (%)				
MPO	78 (76.5)	46 (83.6)	32 (68.1)	85 (85)
PR3	19 (18.6)	9 (16.4)	10 (21.3)	15 (15)
No ANCA	5 (5)	0 (0)	5 (11)	0 (0)
Renal manifestations				
Histologic class, n (%)				
Focal	19 (19)	13 (24)	6 (13)	20 (20)
Mixed	30 (29)	16 (29)	14 (30	41 (41)
Crescentic	19 (19)	8 (15)	11 (23)	23 (23)
Sclerotic	7 (7)	2 (4)	5 (11)	16 (16)
Crescents	37.4 ± 23.7	32.8 ± 24.1	43 ± 22.4	33 ± 24.7
Sclerosis	19.3 ± 19.9	18.4 ± 19.1	20.3 ± 21.1	27 ± 23.0
Normal glomeruli	27.1 ± 25.6	30.2 ± 27.3	23.4 ± 23.3	19 ± 23.0
Extrarrenal manifestations, n (%)	45 (44)	25 (46)	20 (40)	50 (50)
Treatment, n (%)				
Cyclophosphamide IV	61 (60)	35 (64)	26 (55)	43 (43)
Rituximab	22 (22)	20 (36)	2 (4)	42 (42)
Plasmapheresis	18 (18)	10 (18)	8 (17)	29 (29)
Acute HD, n (%)	27 (27)	16 (29)	11 (23)	21 (21)
Serum C3, mg/dl	120 ± 25.2	117 ± 25.1	124 ± 25.4	102 ± 47.6
Serum C4, mg/dl	18.6 ± 10.5	14.2 ± 8.31	27.7 ± 8.81	24.4 ± 13.0
Serum creatinine, mg/dl	4.00 ± 3.00	4.06 ± 3.00	4.03 ± 3.02	4.00 ± 3.30
eGFR, ml/min per 1.73 m^2	23.6 ± 18.9	22.5 ± 17.0	41.3 ± 42.7	23.0 ± 19.0
Proteinuria, g/24 h	0.85 [0.50–1.50]	0.90 [0.50–1.80]	0.80 [0.60–1.80]	1.33 [0.53–2.10]
BVAS index	16 ± 5	16 ± 4	15 ± 5	16 ± 5

AAV, antineutrophil cytoplasmic antibody–associated vasculitis; ANCA, antineutrophil cytoplasmic antibody; BVAS, Birmingham Vasculitis Activity Score; C3, complement C3; eGFR, estimated glomerular filtration rate; HD, hemodialysis; MPO, myeloperoxidase; PR3, proteinase 3.
Data are given as mean ± SD or median [interquartile range] unless otherwise indicated.

Table 2 | Allele frequencies of common gene variant in the *CFH-CFHR* locus, *MCP*, *CFB*, and *C3* in AAV and control populations

Genetic variants		Control[a] frequency	Discovery cohort (n = 99) frequency	OR (95% CI)	*P* value	Validation cohort (n = 100) frequency	OR (95% CI)	*P* value
CFH haplotypes	H1	0.283	0.318	1.180 (0.819–1.699)	0.40	0.355	1.392 (0.974–1.989)	0.078
	H2	0.212	0.177	0.798 (0.518–1.229)	0.34	0.165	0.735 (0.474–1.139)	0.20
	H3	0.210	0.192	0.895 (0.587–1.366)	0.67	0.220	1.063 (0.708–1.597)	0.76
	H4a	0.141	0.141	1.007 (0.622–1.632)	1	0.115	0.795 (0.476–1.326)	0.45
	H4b	0.067	0.051	0.743 (0.355–1.556)	0.48	0.050	0.735 (0.335–1.539)	0.48
	H5	0.028	0.030	1.099 (0.406–2.972)	0.80	0.020	0.718 (0.229–2.253)	0.79
	H6	0.021	0.045	2.249 (0.879–5.755)	0.12	0.030	1.460 (0.523–4.160)	0.58
Deletion (*CFHR3-CFHR1*)		0.234	0.220	0.910 (0.573–1.446)	0.72	0.170	0.691 (0.445–1.073)	0.11
MCP haplotypes	AAT	0.605	0.591	0.945 (0.663–1.347)	0.79	0.570	0.472 (0.610–1.233)	0.87
	GGC	0.280	0.253	0.870 (0.586–1.293)	0.55	0.270	0.953 (0.646–1.406)	0.84
	Other	0.116	0.157	1.417 (0.857–2.343)	0.19	0.160	1.454 (0.883–2.395)	0.15
CFB alleles[b]	Q/W	**0.276**	**0.182**	**0.585 (0.394–0.867)**	**0.008**	**0.180**	**0.577 (0.389–0.856)**	**0.006**
C3 alleles	G	0.201	0.172	0.827 (0.549–1.244)	0.42	0.200	0.997 (0.676–1.470)	1

AAV, antineutrophil cytoplasmic antibody–associated vasculitis; CI, confidence interval; OR, odds ratio.
[a]Controls for *CFH* haplotypes, n = 225; deletion (*CFHR3-CFHR1*), n = 188; *MCP* haplotypes, n = 107; *CFB* alleles, n = 383; and *C3* alleles, n = 389.
[b]CFB alleles R, Q, and W refer to the amino acids at position 32 in the protein.
Bold data indicate statistically significant differences.

Because the activation of the AP may be modulated by the levels of FHR proteins, we also determined the plasma concentration of FHR-1, FHR-2, and FHR-5. As depicted in Figure 3, the levels of these proteins were significantly increased in active AAV compared with controls, and only FHR-1 levels were also different between active and remission samples. The increase in FHR levels, especially FHR-1, suggests that the balance between the AP regulator FH and the FHRs may be compromised during the acute phase of the disease.

The alterations in complement levels observed in the discovery cohort were confirmed in some plasma samples from the validation cohort (Supplementary Figure S2).

In addition to measuring complement levels, we also investigated the presence of autoantibodies to FH and C3 convertase in the discovery cohort. Of the 102 patients, 1 patient had low levels of anti-FH antibodies without being homozygous for the *CFHR3/CFHR1* deletion. Interestingly, this patient had a severe course of the disease, did not reach remission, and required chronic kidney replacement therapy after a month from diagnosis. Antibodies to C3 convertase were only sought in patients with plasma C3 levels below the normal range (n = 23), identifying 5 patients who were positive. Notably, these antibodies did not recognize the independent components of C3 convertase (i.e., C3b and FB) and were identified in the patients with some of the lowest C3 levels (data not shown).

Plasma levels of FH protein family are genetically determined

It has been previously reported that plasma levels of FH and some of the FHRs are to some extent genetically determined. As expected, FHR-1 levels observed in the patients are highly determined by the presence of Δ*CFHR3-CFHR1*, being null in individuals with 2 copies of the deletion, intermediate in heterozygous individuals, and higher in individuals without the deletion (0 vs. 130 ± 55 and 250 ± 79 μg/ml, respectively). In addition, plasma FH levels were also significantly associated with the Δ*CFHR3-CFHR1* genotypes, with patients homozygous for the Δ*CFHR3-CFHR1* presenting higher FH levels compared with the heterozygotes or patients without the deletion (261 ± 47 vs. 216 ± 59 and 199 ± 59 μg/ml; $P = 0.044$ and $P = 0.021$, respectively). This is consistent with previously reported data showing that the *ΔCFHR3-CFHR1* forms part of an extended haplotype that includes the gene encoding FH (*CFH-H4*), a haplotype that has been associated with elevated FH plasma levels.[16]

FHR-1 deficiency protects against severe forms of renal AAV

We then investigated whether complement gene variants or plasma complement levels at diagnosis associate with kidney disease severity. To do so, patients from the AAV active cohort were stratified into 3 groups (T1, T2, and T3) based on tertiles of the urinary levels at disease onset of usCD163, a marker of macrophages that closely correlates with the kidney activity in patients with AAV.[17] Their clinical characteristics are summarized in Supplementary Table S4, where group T1 corresponds to patients with milder forms and group T3 to the most severe forms. Although our results should be taken with caution as only 50 to 53 samples were available for these analyses, both genetic variants and plasma levels of some components were significantly associated with kidney disease severity. Notably, *CFH-H1* haplotype is significantly associated with increased renal severity, whereas *CFH-H2* haplotype and *ΔCFHR3/CFHR1* are associated with milder forms of kidney disease (Table 3; Supplementary Table S4). Consistent with these genetic observations, plasma FHR-1 levels are significantly lower in patients with the lowest usCD163 levels (T1), and hence the FH/FHR-1 ratio is higher (Table 4 and Figure 4a and b). In addition, FH, properdin, and C3 levels are significantly reduced in cases with the highest usCD163 levels, suggesting that there is more complement activation in the severe forms of the disease (Table 4). In line with these observations, FHR-1 levels and the FH/FHR-1 ratio were also significantly

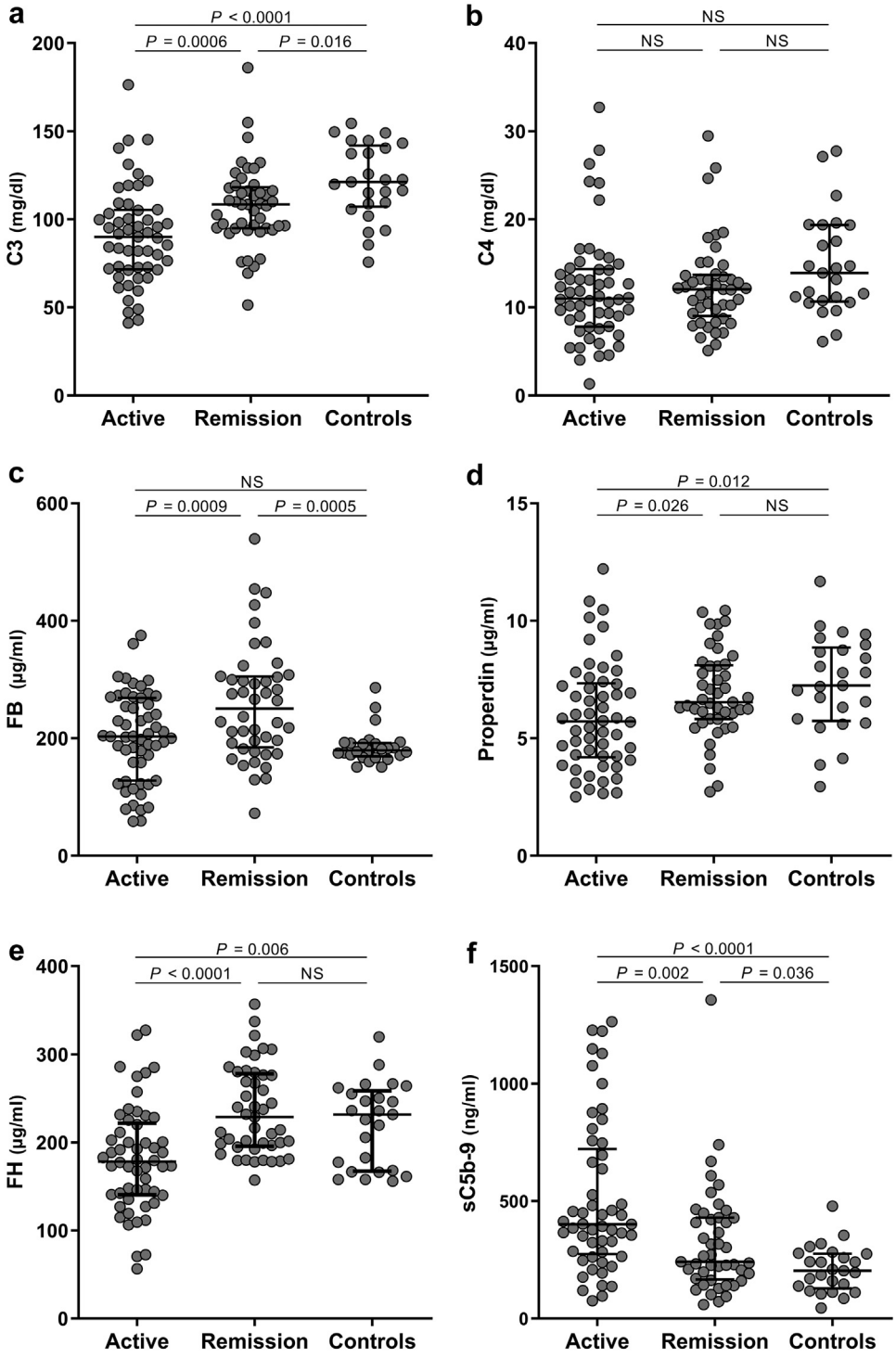

Figure 1 | Plasma levels of complement components in patients with active antineutrophil cytoplasmic antibody–associated vasculitis (n = 54), patients in remission (n = 45), and healthy controls (n = 25). (**a**) Plasma levels of complement C3. (**b**) Plasma levels of complement C4. (**c**) Plasma levels of factor B (FB). (**d**) Plasma levels of properdin. (**e**) Plasma levels of factor H (FH). (**f**) Plasma levels of soluble C5b-9 (sC5b-9). Median and interquartile ranges are depicted, and unpaired *t* test and Mann-Whitney test were applied. NS, not significant.

different between patients with a less severe renal histologic phenotype (focal) and those patients with a more severe phenotype (mixed, crescentic, and sclerotic), according to the Berden classification[18,19] (Figure 4c and d).

Urinary sC5b-9 as a potential marker of kidney disease activity

Given that all our patients had renal involvement, we wondered if urinary sC5b-9 could be used as marker of renal activity. As expected, a significant increase of urinary sC5b-9

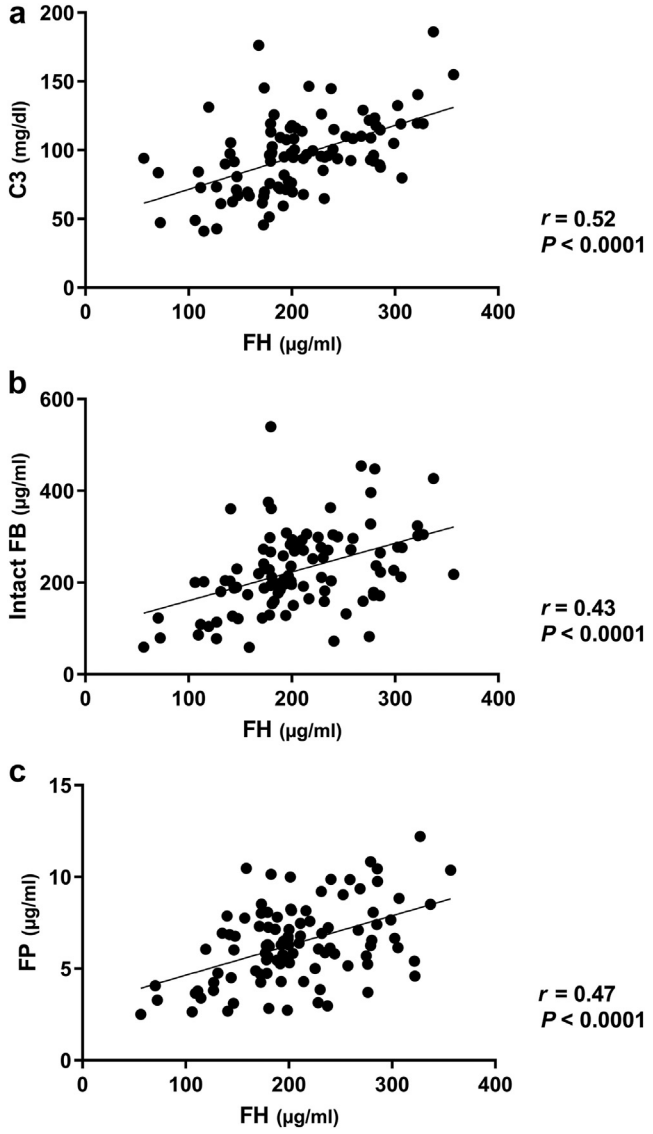

Figure 2 | Positive correlations between circulating levels of factor H (FH), factor B (FB), complement C3, and properdin in patients with active antineutrophil cytoplasmic antibody–associated vasculitis. Correlation between (**a**) FH and C3, (**b**) FH and FB, and (**c**) FH and properdin. Pearson correlation test was applied. FP, factor P.

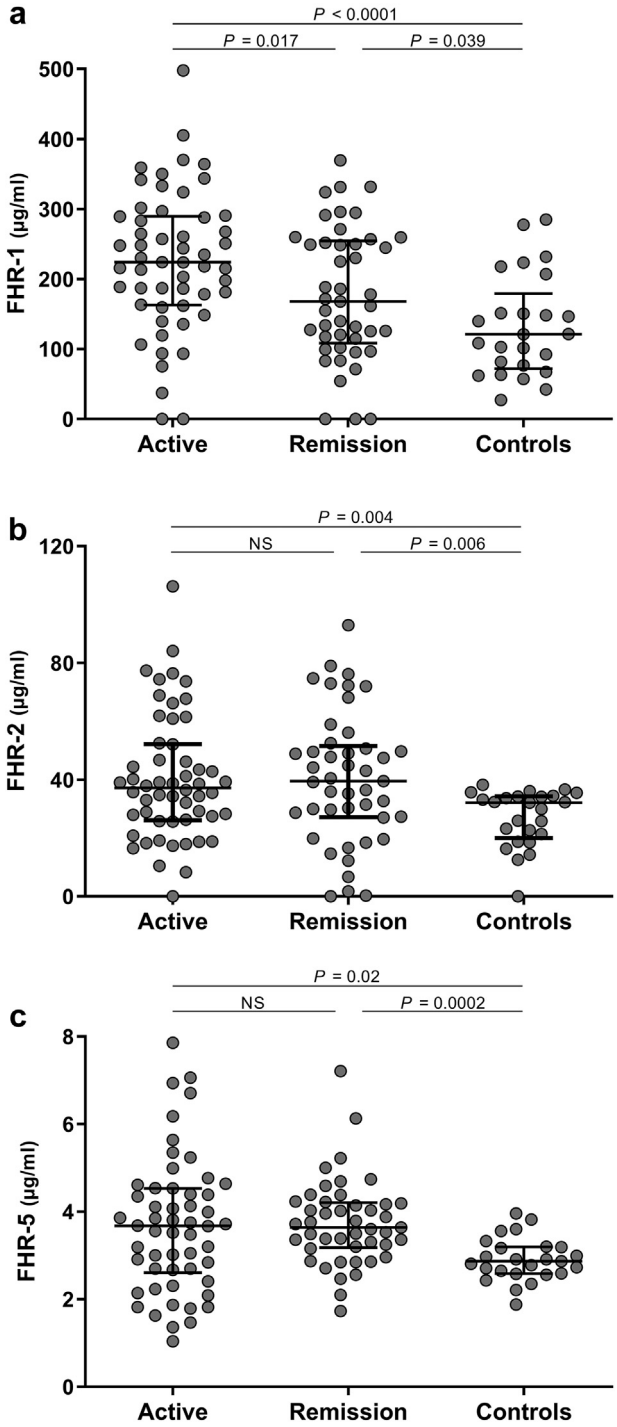

Figure 3 | Plasma levels of factor H–related proteins (FHRs) in patients with active antineutrophil cytoplasmic antibody–associated vasculitis (AAV) (n = 54), patients with remission of AAV (n = 45), and healthy controls (n = 25). (**a**) Plasma levels of FHR-1. (**b**) Plasma levels of FHR-2. (**c**) Plasma levels of FHR-5. Median and interquartile ranges are depicted, and unpaired *t* test was applied. NS, not significant.

in active AAV was observed compared with controls. Interestingly, high levels of urinary sC5b-9 were associated with both more severe histologic features and high levels of usCD163 phenotypes (Figure 5). Notably, no correlation was found between plasma and urine sC5b-9 levels, nor between plasma sC5b-9 and renal histologic classes and usCD163 (data not shown), suggesting that urine sC5b-9 is a better marker of intrarenal complement activation and renal damage than plasma sC5b-9 levels.

Longitudinal analyses of plasma complement components in AAV

A prospective study was performed with the active patients from the discovery cohort. After 12 months of follow-up, 34 patients reached clinical remission, 8 patients did not reach clinical remission, 6 patients died, and 5 patients were lost during the follow-up. During this period, 4 patients had relapsing AAV, and they all responded to the new line of treatment. From the

Table 3 | Allele frequencies of complement gene variants in patients with AAV stratified according to usCD163 tertiles

Genetic variants		usCD163 T1 (n = 18) Frequency	usCD163 T2 (n = 17) Frequency	usCD163 T3 (n = 15) Frequency	*P* value
CFH haplotypes	**H1**	**0.167**	**0.471**	**0.600**	**0.0010**
	H2	**0.306**	**0.118**	**0**	**0.0015**
	H3	0.167	0.147	0.233	0.73
	H4a	0.167	0.059	0.067	0.33
	H4b	0.056	0.059	0	0.39
	H5	0	0.029	0.033	0.57
	H6	0.083	0.059	0	0.27
Deletion (*CFHR3-CFHR1*)	No	0.611	0.706	0.867	0.26
	Het	0.278	0.294	0.133	0.51
	Hom	0.111	0	0	0.16
	Alelle frequency	0.278	0.118	0.063	0.050
MCP haplotypes	AAT	0.583	0.647	0.600	0.85
	GGC	0.222	0.176	0.333	0.33
	Other	0.194	0.176	0.067	0.30
CFB alleles	Q/W	0.333	0.118	0.167	0.068
C3 alleles	G	0.278	0.265	0.133	0.32

AAV, antineutrophil cytoplasmic antibody–associated vasculitis; Het, heterozygous; Hom, homozygous; No, absence of the deletion; T1, tertile 1; T2, tertile 2; T3, tertile 3; usCD163, urinary soluble CD163.
T1: usCD163, <379 ng/mmol; T2: usCD163, 380 to 1405 ng/mmol; T3: usCD163, >1405 ng/mmol. The χ^2 association test was applied.
Bold data indicate statistically significant differences.

clinical point of view, no difference was observed at diagnosis regarding the age, Birmingham Vasculitis Activity Score, and extrarenal manifestations between patients achieving remission and the ones that did not. However, the kidney function at diagnosis was worse in patients who did not achieve remission (estimated glomerular filtration rate, 7.2 vs. 25 ml/min per 1.73 m^2) (Table 5). From the genetic point of view, complement gene variants were not significantly different between patients achieving remission or not (Supplementary Table S3).

Circulating levels of complement components were measured in serial AAV samples available during the 12 months of follow-up. Interestingly, the 8 patients who did not achieve remission presented significantly lower levels of FH, FB, and properdin at diagnosis compared with those achieving remission (Table 5 and Figure 6), suggesting that a higher degree of AP activation at disease onset associates with a worse disease outcome. We next explored the longitudinal variation of plasma complement components during the 12 months of follow-up. In patients achieving remission, C3 levels increased over time after initiating the immunosuppressive treatment, whereas FHR-1 and sC5b-9 decreased (Figure 7a). In the cases where remission was not achieved, renal substitutive treatment was initiated and, hence, plasma samples were limited. FHR-1 levels increased over time in the few cases where the samples were available (Figure 7b).

DISCUSSION

There is now strong evidence that the activation of complement AP is involved in the pathogenesis of AAV. In the present study, we performed a comprehensive analysis of the AP components in a Spanish AAV cohort, and we investigated their impact on determining disease susceptibility, severity, and prognosis. We show, for the first time, to our knowledge, that genetic variants in complement components of the AP associate with protection to develop AAV ($CFB_{32Q/W}$) and with severe forms of the disease (*CFH-H1*, *CFH-H2*, and *ΔCFHR3/1*). As previously reported, we observe an activation of the alternative and terminal complement pathways in circulation during active AAV. We also provide new evidence that a higher degree of AP activation at diagnosis associates with worse disease outcome, whereas elevated plasma FHR-1 levels and lower FH/FHR-1 ratio associate with kidney disease severity. Interestingly, autoantibodies against FH (1%) and the C3 convertase (5%) were identified, although the significance of these antibodies in AAV pathogenesis is currently unknown.

For a long time, the role of the complement system in AAV was overlooked as hypocomplementemia is generally not observed and the characteristic histologic renal lesions are pauci-immune.[1,2] In the past decade, however, studies demonstrated that the levels of complement activated fragments are significantly elevated in active AAV compared with remission or control samples, indicating that complement is

Table 4 | Circulating levels of complement components at diagnosis according to usCD163 tertiles

Complement plasmatic levels	usCD163 T1 (n = 18)	usCD163 T2 (n = 18)	usCD163 T3 (n = 17)	*P* value[a]
FH, μg/ml	198 [173–245]	160 [127–229]	159 [121–197]	**0.014**
FHR-1, μg/ml	180 [103–231]	248 [146–340]	257 [201–333]	**0.0027**
FH/FHR-1 ratio	1.05 [0.80–1.45]	0.73 [0.53–1.21]	0.60 [0.54–0.71]	**0.0011**
FHR-2, μg/ml	31.0 [17.6–38.2]	30.7 [20.4–54.3]	46.2 [39.0–71.3]	**0.0062**
FHR-5, μg/ml	3.50 [2.05–4.18]	3.86 [2.63–5.34]	3.68 [2.51–4.50]	0.30
C5, μg/ml	84.9 [66.8–107.5]	72.00 [60.2–83.3]	85.7 [73.4–102.3]	0.063
sC5b-9, ng/ml	380 [235–780]	390 [280–825]	422 [358–681]	0.96
Properdin, μg/ml	6.82 [5.35–8.26]	4.38 [3.33–7.05]	5.27 [3.72–6.76]	**0.038**
FB, μg/ml	191 [126–269]	197 [120–244]	230 [154–281]	0.61
C3, mg/dl	92.5 [71.3–122.7]	86.5 [70.0–96.1]	72.8 [60.2–90.6]	**0.043**
C4, mg/dl	11.0 [8.70–13.9]	12.0 [5.41–15.1]	10.6 [9.16–14.7]	0.90

C3, complement C3; C4, complement C4; C5, complement C5; FB, factor B; FH, factor H; FHR, factor H–related protein; sC5b-9, soluble c5b-9; T1, tertile 1; T2, tertile 2; T3, tertile 3; usCD163, urinary soluble CD163.
[a]Ordinary 1-way analysis of variance test was applied, except for the FH/FHR-1 ratio, where the Kruskal-Wallis test was used.
Data are given as median [interquartile range]. usCD163 levels are normalized by urine creatinine levels. T1: usCD163, <379 ng/mmol; T2: usCD163, 380 to 1405 ng/mmol; and T3: usCD163, >1405 ng/mmol. Bold data indicate $P < 0.05$.

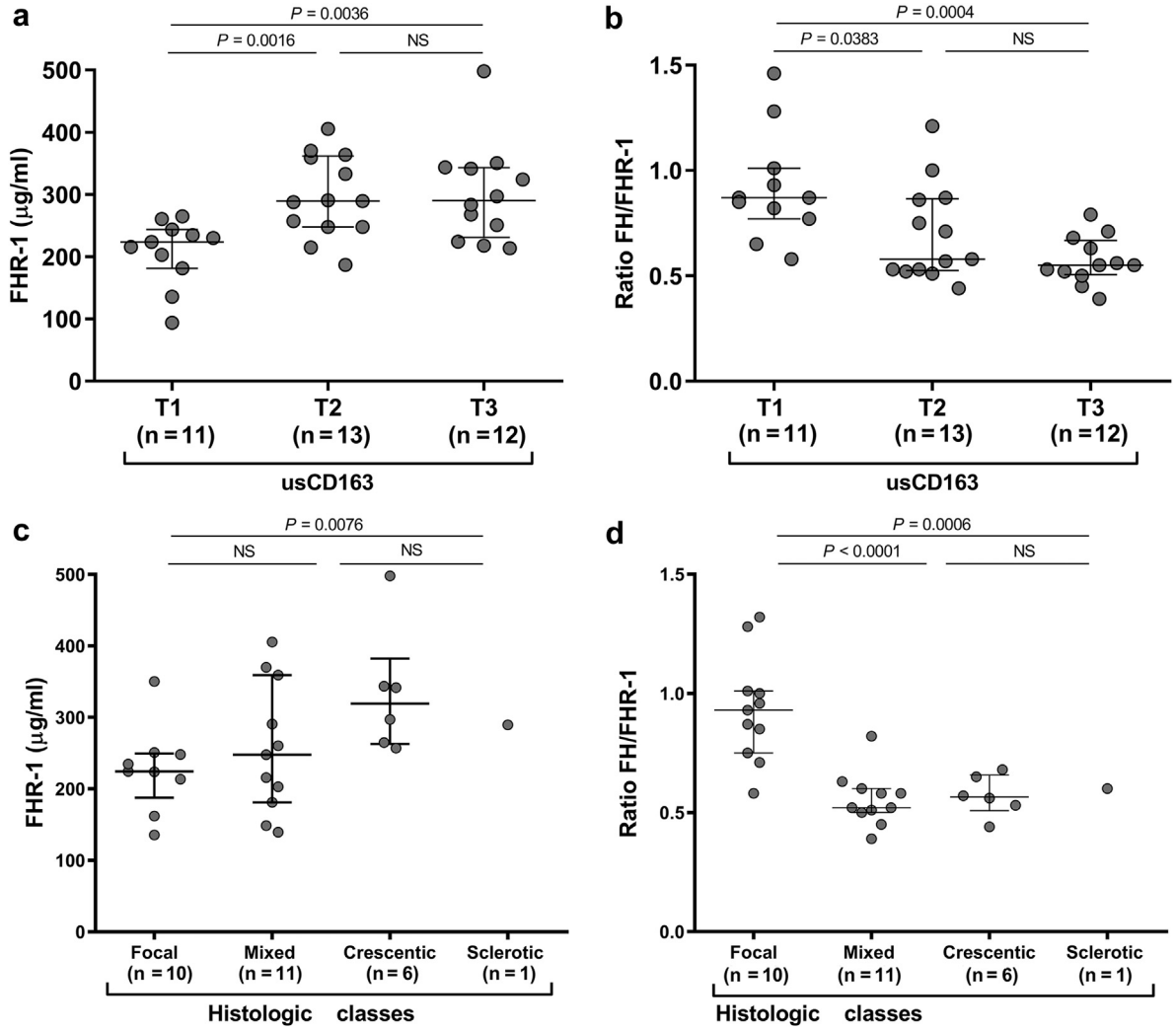

Figure 4 | Plasma factor H (FH)–related protein (FHR)-1 levels and FH/FHR-1 ratio at diagnosis correlate with urinary soluble CD163 (usCD163) tertiles and histologic classes. Only individuals from the active cohort who have both copies of FHR-1 are considered in this study. (**a**) FHR-1 levels and (**b**) FH/FHR-1 ratio in patients stratified into 3 groups, according to usCD163 tertiles (tertile 1 [T1]: usCD163, <379 ng/mmol; tertile 2 [T2]: usCD163, 380–1405 ng/mmol; tertile 3 [T3]: usCD163, >1405 ng/mmol). (**c**) FHR-1 levels and (**d**) FH/FHR-1 ratio in patients stratified by histologic classes. Median and interquartile ranges are depicted, and unpaired *t* test was applied. NS, not significant.

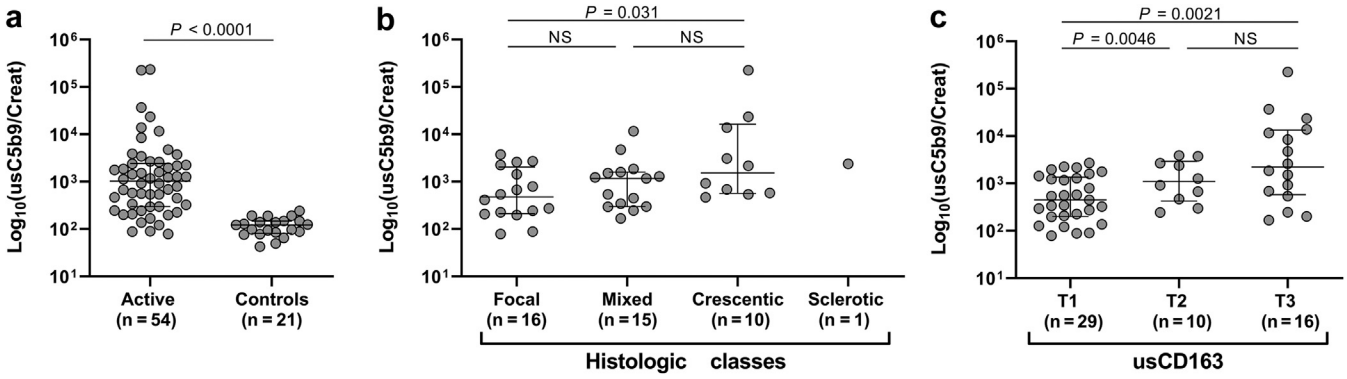

Figure 5 | Urinary soluble C5b-9 (usC5b-9) levels are elevated in active antineutrophil cytoplasmic antibody–associated vasculitis and are associated with kidney disease activity and histologic damage. (**a**) usC5b-9 levels at diagnosis in patients from the discovery active cohort compared with control samples. usC5b-9 levels are stratified according to the (**b**) histologic class (focal vs. mixed, crescentic, and sclerotic) or (**c**) urinary soluble CD163 (usCD163) tertiles. usC5b-9 levels expressed as ng/mmol creatinine (Creat). NS, not significant.

Table 5 | Baseline characteristics of active cohort patients with AAV achieving remission or not

Variable	Remission (n = 34)	No remission (n = 8)	*P* value
Age at diagnosis, yr	68.41 ± 10.54	71.13 ± 16.91	0.57
Male sex, n (%)	22 (65)	5 (62)	0.60
Renal manifestations			
Glomerular crescents, %	33.97 ± 25.87	30.0 ± 14.57	0.74
Sclerotic glomeruli	19.23 ± 18.61	21.71 ± 20.81	0.76
Normal glomeruli	33.94 ± 27.11	25.43 ± 23.35	0.45
Extrarrenal manifestations, n (%)	26 (47)	2 (25)	0.20
Serum creatinine, mg/dl	3.32 ± 2.27	7.79 ± 3.65	<0.0001
eGFR, ml/min per 1.73 m^2	25 ± 15.82	7.17 ± 3.62	0.003
Proteinuria, g/24 h	1.05 ± 0.81	4.88 ± 8.09	0.009
BVAS index	16 ± 4	15 ± 3	0.73
Plasma complement levels			
FH, μg/ml	193 [163–236]	124 [82–166]	**0.0006**
FHR-1, μg/ml	239 [175–304]	201 [130–223]	0.14
FH/FHR-1 ratio	0.85 [0.58–1.28]	0.58 [0.5–0.79]	0.075
FHR-2, μg/ml	38 [25–52]	34 [13–57]	0.72
FHR-5, μg/ml	3.8 [2.7–4.6]	2.9 [2.0–3.7]	0.17
C5, μg/ml	81 [67–104]	79 [67–113]	0.99
sC5b-9, ng/ml	408 [256–818]	377 [136–487]	0.40
Properdin, μg/ml	5.8 [4.7–7.3]	3.7 [3.1–6.4]	**0.026**
FB, μg/ml	213 [168–270]	148 [81–201]	**0.037**
C3, mg/dl	120 [100–182]	129 [99–135]	0.90
C4, mg/dl	11.6 [9.95–19.2]	10.1 [5.60–16.2]	0.18

AAV, antineutrophil cytoplasmic antibody–associated vasculitis; BVAS, Birmingham Vasculitis Activity Score; C3, complement C3; C4, complement C4; C5, complement C5; eGFR, estimated glomerular filtration rate; FB, factor B; FH, factor H; FHR, factor H–related protein; sC5b-9, soluble C5b-9.
Data are given as mean ± SD or median [interquartile range] unless otherwise indicated. Bold data indicate $P < 0.05$.

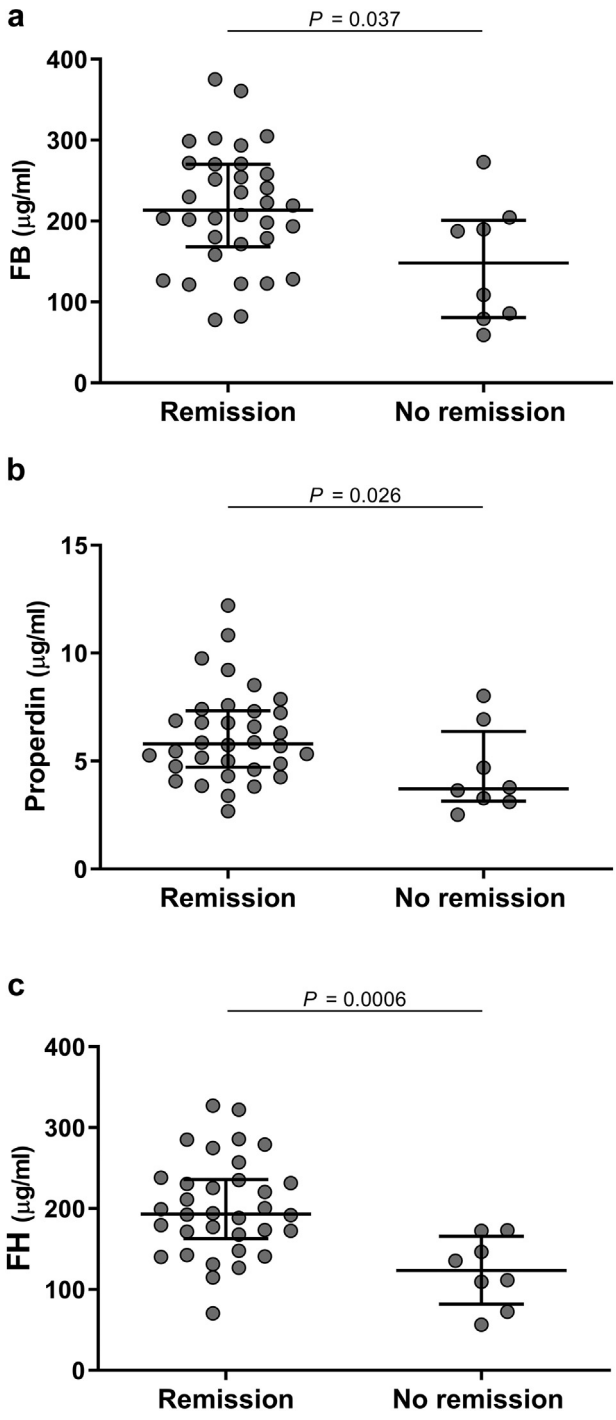

Figure 6 | Plasma levels of factor H (FH), factor B (FB), and properdin at diagnosis were significantly lower in patients who did not achieve remission compared with patients in remission. Remission was assessed at month 12 after diagnosis. (**a**) Plasma levels of FB. (**b**) Plasma levels of properdin. (**c**) Plasma levels of FH. Statistical significance was calculated by Mann-Whitney test.

activated during the active phase of the disease.[3,20] In our study, we also observe evidence of complement activation, as the median levels of C3, FH, FB, properdin, and sC5b-9 were significantly altered in samples with active disease compared with samples in remission. Moreover, we showed a positive correlation between the levels of components of the C3 convertase (C3, FB, and properdin) and FH, which clearly illustrates the activation of the AP during active AAV. Conversely, the median concentration of sC5b-9 was significantly higher in active samples compared with remission samples, evidencing the activation of the terminal pathway during the active phase of the disease.

In other complement-mediated diseases in which the AP is involved, it has been shown that the regulatory function of FH may be compromised by certain FH-related proteins and that the balance between these proteins determines the outcome of complement activation and disease susceptibility.[11,13,21–25] For instance, the *ΔCFHR3/1* has been associated with protection to IgA nephropathy and age-related macular degeneration, and more recently to C3 glomerulopathy, suggesting that the absence of FHR-1 and/or FHR-3 favors complement regulation by FH.[23,26,27] Conversely, elevated FHR-1, FHR-3, FHR-4, and FHR-5 levels have been associated with increased disease susceptibility and worse prognosis in various complement-mediated diseases.[23,25,27–29] We have shown here that circulating FHR-1, FHR-2, and FHR-5 levels are significantly increased in AAV samples compared with

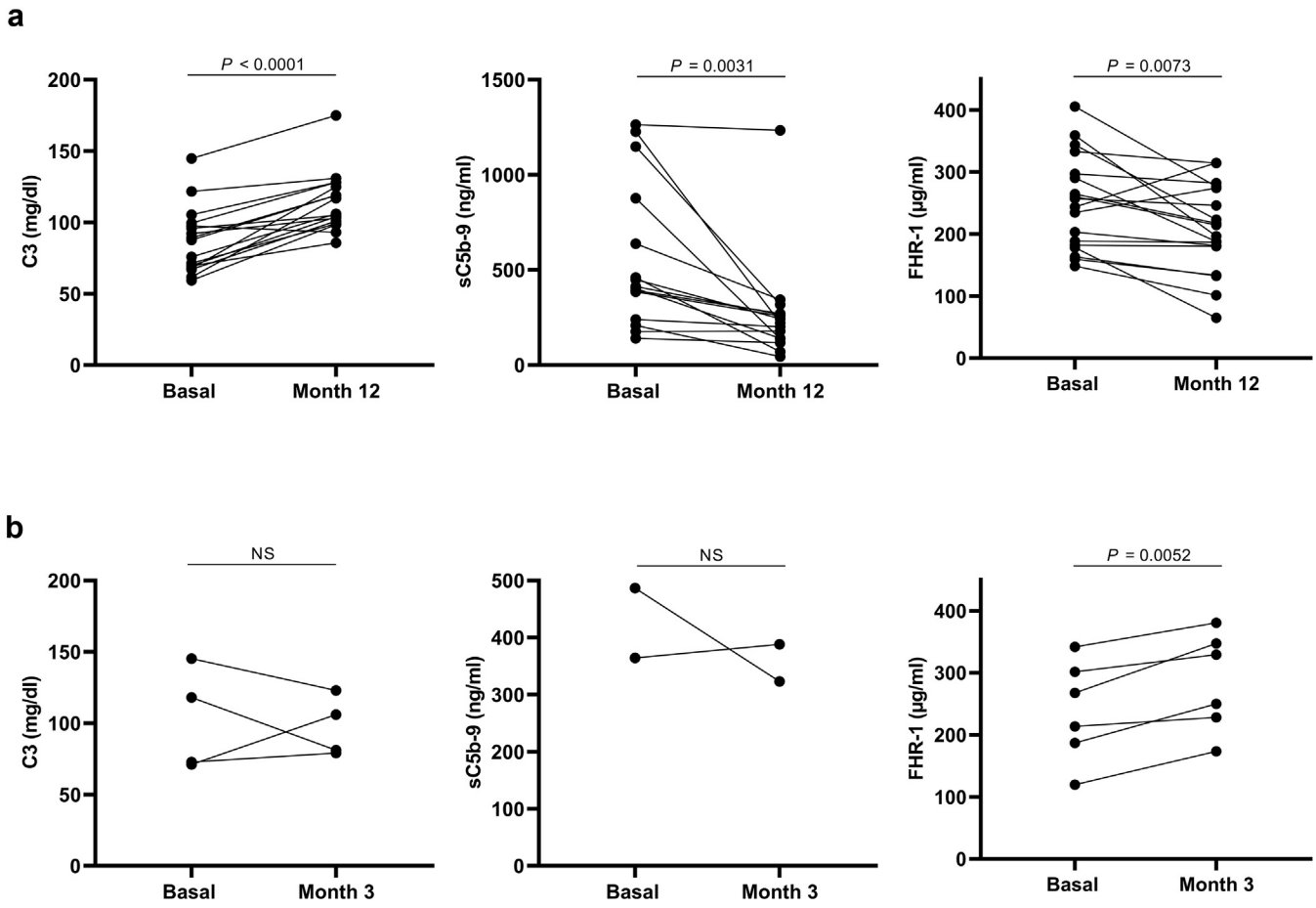

Figure 7 | In longitudinal follow-up, plasma complement C3, soluble C5b-9 (sC5b-9), and factor H–related protein (FHR)-1 levels normalize over time in patients achieving remission. A prospective study was performed in the patients with active antineutrophil cytoplasmic antibody–associated vasculitis (n = 54). (**a**) Circulating C3, sC5b-9, and FHR-1 levels at the time of diagnosis and after 12 months were assessed in patients achieving remission. (**b**) Circulating C3, sC5b-9, and FHR-1 levels at the time of diagnosis and after 3 months were assessed in patients who did not respond. Statistical differences were determined using the paired *t* test or the Wilcoxon matched-pairs signed rank test. NS, not significant.

controls, suggesting that these proteins may also be involved in AAV pathogenesis.

Important findings from our report derive from the study of complement levels in longitudinal samples, which showed that the highest level of AP activation at diagnosis is associated with worse disease outcome. In addition, C3, sC5b-9, and FHR-1 were useful markers of disease activity as their levels normalized over time in patients achieving remission. However, in the few available samples of patients who did not reach remission, C3, sC5b-9, and FHR-1 levels either did not normalize or were even higher.

It is well known that common gene variants in complement genes associate with either increased susceptibility or protection to develop a wide range of pathologic conditions.[30] In this study, we show that the *CFB*$_{32Q/W}$ variants are significantly decreased in patients with AAV, suggesting that these alleles are protective factors for the development of AAV. FB$_{32Q/W}$ variants are known to be less active molecules compared with the variant FB$_{32R}$, as they show less potential to form the C3 convertase and to amplify complement activation.[31,32] Interestingly, in our cohort with AAV, there are no patients homozygous for *CFB*$_{32Q/W}$. The fact that the less active FB molecules are a protective factor in AAV indicates that the lower the capacity to activate the complement AP the better for preventing the disease. Notably, FB$_{32Q/W}$ variants were previously associated with protection to age-related macular degeneration, a disease that is also characterized by dysregulation of the AP.[33]

In addition to the association of *CFB* variants with AAV susceptibility, we identified other important genotype-phenotype correlations. Variants in genes of the FH family determine the severity of kidney disease in AAV, as measured by usCD163. The *CFH* haplotype H1, previously associated with increased risk to age-related macular degeneration and C3 glomerulopathy,[30,34,35] is significantly associated with increased risk to develop severe renal damage. Conversely, the haplotype *CFH-H2*, which encodes an FH with higher capacity to regulate complement, is associated with protection to several complement-mediated diseases[30,34,35] and, in our study, is significantly associated with reduced risk to develop severe kidney disease in AAV. Although the absence of *ΔCFHR3/1* did not

reach statistical significance in severe forms of AAV, this genetic observation is supported by the fact that elevated levels of FHR-1 were also significantly associated with the severity of the disease in our cohort. Interestingly, in another cohort with AAV with renal involvement, it was reported that patients with *ΔCFHR3/1* had less fibrinoid necrosis and less cellular proliferation compared with patients without the deletion.[36] Our study cohorts are predominantly myeloperoxidase-associated AAV with renal involvement, and it will be interesting to see if the same genetic associations are observed in other populations with AAV, such as proteinase 3–associated AAV or AAV without renal manifestations.

Despite the fact that the exact molecular mechanisms behind the complement genetic associations with AAV are unknown, our data support that common complement genetic variants involving the FH protein family determine the outcome of complement activation within the kidney and the development of severe forms of kidney disease in AAV. In this context, the balance between FH and FHR-1 is essential, and both quantitative and qualitative variations in these proteins influence the outcome of AP activation in AAV. Interestingly, FHR-1 has been recently proposed as an inducer of sterile inflammation by the activation of the inflammasome NLRP3 (nucleotide-binding oligomerization domain, leucine-rich repeat receptor and pyrin-domain-containing 3) in monocytes, with higher FHR-1 levels correlating with lower glomerular infiltration rates and increased interleukin-1β serum levels in patients with AAV.[36,37] Moreover, we also reported that surface-bound FHR-1 promotes AP activation and compromises the regulatory function of FH.[27] Whether the detrimental role of FHR-1 in AAV is due to the promotion of complement activation, the induction of the NLRP3 inflammasome, or both is not known, and further studies will be needed to clarify this point.

In summary, we showed, for the first time, to our knowledge, that complement gene variants in components of the AP predispose to AAV and are associated with kidney disease severity in patients with AAV. The clear involvement of complement in AAV pathogenesis makes targeting complement components an attractive therapeutic approach. In fact, avacopan, an oral C5a receptor antagonist, is currently being used for the treatment of AAV and has been proven to be superior to the conventional glucocorticoids.[7] Our data also support that the inhibition of components of the AP, such as FB and FHR-1, may be a useful therapeutic target in AAV. This is particularly important given the availability of drugs that target AP components, such as pegcetacoplan, iptacopan, or danicopan.[38]

DISCLOSURE

EGdJ reports receiving lecture fees from Alexion Pharmaceuticals and Astellas; and consultancy fees from Q32Bio. GF-J has received honoraria from GlaxoSmith Kline for scientific advisory board fees. FC-F reports receiving research funding from, and serving on a speaker's bureau for, Novartis Pharmaceuticals. MP reports having consultancy agreements with Alexion, AstraZeneca, GlaxoSmithKline, Novartis, Silence, and Travere; receiving honoraria from Alexion, GlaxoSmithKline, Novartis, and Travere; and receiving research funding from Novartis. All the other authors declared no competing interests.

DATA STATEMENT

All data are included in the article and/or supporting materials. Additional information can be provided on request to the principal investigators of the study.

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AUTHOR CONTRIBUTIONS

GF-J, JV, and EGdJ designed the study; BM-T set up enzyme-linked immunosorbent assay methods for factor H–related protein (FHR)-1, FHR-2, and FHR-5 quantifications. LL-C and LJ-V performed genetic studies and complement protein determinations. GF-J, MA, TC, LSC, FC-F, MP, JD, PA-P, and JV collected the patient samples and the clinical data. LL-C, GF-J, and EGdJ collected and analyzed all data. LL-C, GF-J, and EGdJ wrote the manuscript, and all coauthors revised and approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Supplementary Methods.

Supplementary Figure S1. Description of single-nucleotide polymorphisms used to build the 7 most common *CFH* haplotypes.

Supplementary Figure S2. Plasma complement determinations in the validation cohort.

Supplementary Table S1. Description of capture, primary, and secondary antibodies used in the sandwich enzyme-linked immunosorbent assays (ELISAs) to quantify complement components.

Supplementary Table S2. Circulating levels of complement components in active and remission antineutrophil cytoplasmic antibody–associated vasculitis (AAV), and control samples.

Supplementary Table S3. Association analysis of common complement gene variants in patients with antineutrophil cytoplasmic antibody–associated vasculitis (AAV) achieving remission or not.

Supplementary Table S4. Clinical and histopathologic data of patients with active antineutrophil cytoplasmic antibody–associated vasculitis (AAV) stratified according to urinary soluble CD163 (usCD163) tertiles.

REFERENCES

1. Jennette JC, Falk RJ, Hu P, Xiao H. Pathogenesis of antineutrophil cytoplasmic autoantibody-associated small-vessel vasculitis. *Annu Rev Pathol*. 2013;8:139–160.
2. Falk RJ, Jennette JC. ANCA small-vessel vasculitis. *J Am Soc Nephrol*. 1997;8:314–322.
3. Gou SJ, Yuan J, Chen M, et al. Circulating complement activation in patients with anti-neutrophil cytoplasmic antibody-associated vasculitis. *Kidney Int*. 2012;83:129–137.
4. Villacorta J, Diaz-Crespo F, Acevedo M, et al. Glomerular C3d as a novel prognostic marker for renal vasculitis. *Hum Pathol*. 2016;56:31–39.
5. Xiao H, Schreiber A, Heeringa P, et al. Alternative complement pathway in the pathogenesis of disease mediated by anti-neutrophil cytoplasmic autoantibodies. *Am J Pathol*. 2007;170:52–64.
6. Xiao H, Dairaghi DJ, Powers JP, et al. C5a receptor (CD88) blockade protects against MPO-ANCA GN. *J Am Soc Nephrol*. 2014;25:225–231.
7. Jayne DRW, Merkel PA, Schall TJ, et al. Avacopan for the treatment of ANCA-associated vasculitis. *N Engl J Med*. 2021;384:599–609.
8. Lucientes-Continente L, Marquez-Tirado B, Goicoechea de Jorge E. The factor H protein family: the switchers of the complement alternative pathway. *Immunol Rev*. 2023;313:25–45.
9. Csincsi AI, Kopp A, Zoldi M, et al. Factor H-related protein 5 interacts with pentraxin 3 and the extracellular matrix and modulates complement activation. *J Immunol*. 2015;194:4963–4973.
10. Csincsi AI, Szabo Z, Banlaki Z, et al. FHR-1 binds to C-reactive protein and enhances rather than inhibits complement activation. *J Immunol*. 2017;199:292–303.
11. Goicoechea de Jorge E, Caesar JJ, Malik TH, et al. Dimerization of complement factor H-related proteins modulates complement activation *in vivo*. *Proc Natl Acad Sci U S A*. 2013;110:4685–4690.
12. Hebecker M, Jozsi M. Factor H-related protein 4 activates complement by serving as a platform for the assembly of alternative pathway C3 convertase via its interaction with C3b protein. *J Biol Chem*. 2012;287:19528–19536.
13. Jozsi M, Tortajada A, Uzonyi B, et al. Factor H-related proteins determine complement-activating surfaces. *Trends Immunol*. 2015;36:374–384.
14. Luqmani RA, Bacon PA, Moots RJ, et al. Birmingham Vasculitis Activity Score (BVAS) in systemic necrotizing vasculitis. *QJM*. 1994;87:671–678.
15. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150:604–612.
16. Lores-Motta L, van Beek AE, Willems E, et al. Common haplotypes at the CFH locus and low-frequency variants in CFHR2 and CFHR5 associate with systemic FHR concentrations and age-related macular degeneration. *Am J Hum Genet*. 2021;108:1367–1384.
17. Villacorta J, Lucientes L, Goicoechea E, et al. Urinary soluble CD163 as a biomarker of disease activity and relapse in antineutrophil cytoplasm antibody-associated glomerulonephritis. *Clin Kidney J*. 2021;14:212–219.
18. Berden AE, Ferrario F, Hagen EC, et al. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol*. 2010;21:1628–1636.
19. Diaz-Crespo F, Villacorta J, Acevedo M, et al. The predictive value of kidney biopsy in renal vasculitis: a multicenter cohort study. *Hum Pathol*. 2016;52:119–127.
20. Moiseev S, Lee JM, Zykova A, et al. The alternative complement pathway in ANCA-associated vasculitis: further evidence and a meta-analysis. *Clin Exp Immunol*. 2020;202:394–402.
21. Goicoechea de Jorge E, Tortajada A, Garcia SP, et al. Factor H competitor generated by gene conversion events associates with atypical hemolytic uremic syndrome. *J Am Soc Nephrol*. 2018;29:240–249.
22. Poppelaars F, Goicoechea de Jorge E, Jongerius I, et al. A family affair: addressing the challenges of factor H and the related proteins. *Front Immunol*. 2021;12:660194.
23. Tortajada A, Gutierrez E, Goicoechea de Jorge E, et al. Elevated factor H-related protein 1 and factor H pathogenic variants decrease complement regulation in IgA nephropathy. *Kidney Int*. 2017;92:953–963.
24. Tortajada A, Yebenes H, Abarrategui-Garrido C, et al. C3 glomerulopathy-associated CFHR1 mutation alters FHR oligomerization and complement regulation. *J Clin Invest*. 2013;123:2434–2446.
25. Medjeral-Thomas NR, Lomax-Browne HJ, Beckwith H, et al. Circulating complement factor H-related proteins 1 and 5 correlate with disease activity in IgA nephropathy. *Kidney Int*. 2017;92:942–952.
26. Hughes AE, Orr N, Esfandiary H, et al. A common CFH haplotype, with deletion of CFHR1 and CFHR3, is associated with lower risk of age-related macular degeneration. *Nat Genet*. 2006;38:1173–1177.
27. Marquez-Tirado B, Gutierrez-Tenorio J, Tortajada A, et al. Factor H-related protein 1 drives disease susceptibility and prognosis in C3 glomerulopathy. *J Am Soc Nephrol*. 2022;33:1137–1153.
28. Cipriani V, Lores-Motta L, He F, et al. Increased circulating levels of factor H-related protein 4 are strongly associated with age-related macular degeneration. *Nat Commun*. 2020;11:778.
29. Pouw RB, Gomez Delgado I, Lopez Lera A, et al. High complement factor H-related (FHR)-3 levels are associated with the atypical hemolytic-uremic syndrome-risk allele CFHR3*B. *Front Immunol*. 2018;9:848.
30. Goicoechea de Jorge E, Lopez Lera A, Bayarri-Olmos R, et al. Common and rare genetic variants of complement components in human disease. *Mol Immunol*. 2018;102:42–57.
31. Montes T, Tortajada A, Morgan BP, et al. Functional basis of protection against age-related macular degeneration conferred by a common polymorphism in complement factor B. *Proc Natl Acad Sci U S A*. 2009;106:4366–4371.
32. Shi DC, Feng SZ, Zhong Z, et al. Functional variant rs12614 in CFB confers a low risk of IgA nephropathy by attenuating complement alternative pathway activation in Han Chinese. *Front Immunol*. 2022;13:973169.
33. Loyet KM, Deforge LE, Katschke KJ Jr, et al. Activation of the alternative complement pathway in vitreous is controlled by genetics in age-related macular degeneration. *Invest Ophthalmol Vis Sci*. 2012;53:6628–6637.
34. Pickering MC, Goicoechea de Jorge E, Martinez-Barricarte R, et al. Spontaneous hemolytic uremic syndrome triggered by complement factor H lacking surface recognition domains. *J Exp Med*. 2007;204:1249–1256.
35. Rodriguez de Cordoba S. Genetic variability shapes the alternative pathway complement activity and predisposition to complement-related diseases. *Immunol Rev*. 2023;313:71–90.
36. Skerka C, Pradel G, Halder LD, et al. Factor H-related protein 1: a complement regulatory protein and guardian of necrotic-type surfaces. *Br J Pharmacol*. 2021;178:2823–2831.
37. Irmscher S, Brix SR, Zipfel SLH, et al. Serum FHR1 binding to necrotic-type cells activates monocytic inflammasome and marks necrotic sites in vasculopathies. *Nat Commun*. 2019;10:2961.
38. Lamers C, Ricklin D, Lambris JD. Complement-targeted therapeutics: an emerging field enabled by academic drug discovery. *Am J Hematol*. 2023;98(suppl 4):S82–S89.