C5 nephritic factors drive the biological phenotype of C3 glomerulopathies

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C3 Glomerulopathies, which include Dense Deposit Disease and C3 Glomerulonephritis, are associated with genetic and acquired dysregulation of the C3 convertase alternative pathway of complement. The potential role of the activation of the C5 convertase has not been studied extensively. Here we analyzed IgG samples from patients with C3 Glomerulopathies to identify circulating autoantibodies that stabilize the C3 alternative pathway (C3 Nephritic Factors) as well as C5 convertases (C5 Nephritic Factors), thus preventing decay of these enzyme complexes. Rare variants in alternative pathway genes were found in 28 of 120 tested patients. C3 and C5 Nephritic Factors were found in 76 of 101 (75%) and 29 of 59 (49%) of the patients, respectively. Therefore, we compared the results of the assays for the C3 and C5 nephritic factors functional activity: 29% were positive for C3 Nephritic Factors alone, 39% were positive for both C3 and C5 Nephritic Factors, and 10% were positive for C5 Nephritic Factors alone. We found that the addition of properdin-enhanced stabilization of C3 convertase in the presence of IgG doubly positive for both Nephritic Factors, while it did not modify the stabilization mediated by IgG solely positive for C3 Nephritic Factors. Both C3 and C5 Nephritic Factors correlated with C3 consumption, while only C5 Nephritic Factors correlated with sC5b9 levels. C5 Nephritic Factors–positive patients were more likely to have C3 Glomerulonephritis than Dense Deposit Disease. Thus, dysregulation of the C5 convertase contributes to C3 Glomerulopathies inter-disease differences and may have direct therapeutic implications.

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The C3 glomerulopathies (C3G) are chronic renal diseases characterized by glomerular lesions with predominant complement component C3 fragment deposition.[1,2] The 2 major subgroups of C3G, dense deposit disease (DDD) (also known as membranoproliferative glomerulonephritis type 2) and C3 glomerulonephritis (C3GN), are caused by dysregulation of the alternative pathway (AP) of the complement system due to genetic and acquired factors.[3] Although mutations have been identified in several complement genes, in >50% of the cases, the disease is linked to the presence of AP C3 convertase autoantibodies, the C3 nephritic factors (C3NeFs).[4] The AP C3 convertase is formed after the binding of factor B (FB) to C3b in an Mg^{2+}-dependent manner, followed by cleavage of FB by factor D (FD).[5] The enzyme C3bBb has a very limited half-life. Properdin binds and stabilizes the C3bBb convertase (C3bBbP) and facilitates the switch of the C3 convertase to the C5 convertase.[6,7] C3NeFs are IgGs that increase the AP activity and exert a stabilizing effect on both the fluid phase and the cell-bound C3 convertases.[8] The complement biomarkers associated with C3NeFs are heterogeneous. In some patients, C3NeF is associated with C3 consumption in the fluid phase but does not always enhance C5 consumption and terminal pathway activation.[9–11] Despite published cases of the association of properdin-independent C3NeF with DDD and properdin-dependent C3NeF with MPGN type I,[12–14] the relationship between properdin, C3NeF, and C3G remains unclear.[15,16] Numerous studies have explored mechanisms of action of C3NeF on the C3bBb, but few have investigated the reactivity of patient IgG to C3bBb and C3bBbP.[17,18]

In this retrospective study, we confirmed that C3G is an acquired rather than a hereditary disease. We identified for the first time patients with circulating autoantibodies stabilizing the C5 convertase, namely, C5NeF, and we showed a correlation between the capacity of patients' IgG to stabilize the C5 convertase and the sC5b-9 levels in patient plasma. We analyzed the autoantibodies profile against the C3 and C5 convertases and showed that C5NeFs are strongly associated with C3GN.

RESULTS

Clinical data and recommended complement analyses

The clinical and biological data in the 127 patients with C3G are summarized in Table 1. The age at onset ranged from 1 month to 72 years, and 57 patients had a pediatric onset (44.9%). Nephrotic syndrome was the most common feature at diagnosis (55.1%). Sixty-three percent of patients were receiving conventional immunosuppressive treatment (corticosteroids, cyclophosphamide, azathioprine, mycophenolate mofetil). At last follow up, end-stage renal disease (ESRD) had developed in 52%. No significant differences were observed in the type of clinical presentation between patients with pediatric and adult onset, but the incidence of ESRD or death was significantly higher in patients with adult onset. At the time of the first laboratory investigation, 53 of 127 patients (41.7%) had a low C3 level and a normal C4, suggesting AP consumption, and 71 of 118 patients (60.2%) had an sC5b-9 level above the normal range, suggesting activation of the terminal complement pathway. The frequency of patients with 1 ($N = 27$) or 2 ($N = 1$) variants was 23.3% (28/120 tested patients). We identified 10 novel and 16 rare variants with a minor allele frequency <1% in 6 complement genes (CFH, CFI, MCP, C3, CFB, CFHR5). Variants were demonstrated as pathogenic in 50% of the cases (13/26). Details of the detected novel or rare variants are shown in minor allele frequency (Supplementary Table S1). The frequency of variants was not significantly different between the patients with pediatric (11/55) and adult (17/65) onset of the disease. Using the cell-bound C3bBb stabilization assay, 75% were positive for C3NeF (76/101) (Supplementary Figure S1A). The stabilization of the C3 convertase was IgG dose dependent (Supplementary Figure S1B/C).

C3NeF activity was found in 72% and 57% of patients with pediatric and adult onset, respectively. Anti-CFH

Table 1 | Clinical, biological, and genetic data according to pediatric or adult onset

Clinical description and biological and genetic results	All	Pediatric	Adult	*P* Value
N (%)	127	57 (44.9)	70 (55.1)	
Sex (M/F) (% M)	72/55 (57)	28/29 (49)	44/26 (62)	0.1504
At diagnosis				
DDD (%)	46 (36)[a]	26 (57)	20 (43)	0.0633
C3GN (%)	81 (64)[a]	31 (38)	50 (62)	0.0633
Age (yr), mean ± SD	23.2 ± 16.8	9.4 ± 4.3	34.3 ± 14.8	
Nephrotic syndrome (%)	59/117 (55)	30/54 (55)	29/63 (46)	0.3556
Acute renal failure (<60 ml/min per 1.73 m^2) (%)	35/99 (35)	16/46 (34.7)	19/53 (35)	1.0000
eGFR (ml/min per 1.73 m^2)	70.8 ± 33.2 ($N = 98$)	74.2 ± 35.1 ($N = 45$)	62.1 ± 30.7 ($N = 53$)	0.0718
Immunosuppressive treatment (%)	65/102 (63)	35/47 (74)	30/55 (54)	**0.0415**
Follow-up, yr	9.8 ± 8.8	8 ± 8.5	12 ± 8.8	0.0108
Time between diagnosis and screening, mo.	124 ± 139	138 ± 157	113 ± 124	0.3179
At last follow-up				
Death	5	0	5	
Dialysis (%)	66/127 (52)	23/57 (40)	43/70 (61)	0.0211
Age at dialysis (yr), mean ± SD	35.3 ± 16.4	23.7 ± 8.7	41.5 ± 16.2	**<0.0001**
Duration of evolution until ESRD (yr) if ESRD, mean ± SD	8 ± 7.7	12.4 ± 8.7	5.7 ± 6	**<0.0001**
Complement component assessment				
C3 (660–1250 mg/l)	726 ± 343	712 ± 358	729 ± 332	0.7822
Low C3 (<660 mg/l) (%)	53/127 (41.7)	24/57 (42)	29/70 (41)	1.0000
C4 (93–380 mg/l)	232 ± 91	228 ± 102	238 ± 82	0.5413
Low C4 (<90 mg/l) (%)	3/127 (2.4)	3/57 (5.3)	0/70 (0)	0.0878
Low factor H (<338 mg/l) (%)	6/127 (4.7)	3/57 (5.3)	3/70 (4.3)	1.0000
Low factor I (<42 mg/l) (%)	3/127 (2.4)	2/57 (3.5)	1/70 (1.4)	1.0000
High sC5b9 (>440 µg/l) (%)	71/118 (60.2)	30/54 (55.5)	41/64 (64.0)	0.3470
Antibodies against complement proteins				
Circulating C3NeF against the C3bBb	80/125 (64)	41/57 (71.9)	39/68 (57.3)	0.0970
Anti-FH Ab	14/116 (12)	9/53 (17)	5/63 (8)	0.1363
Anti-C3 Ab	3/116 (2.6)	1/53 (1.9)	2/63 (3.2)	0.6633
Anti-Fb Ab	3/116 (2.6)	1/53 (1.9)	2/63 (3.2)	0.6633
In association with C3NeF	11/17 (64.7)	7/8 (87.5)	4/9 (44.4)	0.6372
Novel or rare variants in complement genes				
All	28/120 (23.3)	11/55 (20)	17/65 (26.1)	0.4271
With C3NeF	12/28 (42.8)	6/11 (54.5)	6/17 (35.3)	0.3147

Ab, antibody; DDD, dense deposit disease; ESRD, end-stage renal disease; F, female; FH, factor H; M, male.
Pediatric (younger than 18 years of age).
[a]The histologic characteristics have been reported in 85 patients[4] (56 C3GN and 29 DDD patients).
P values in bold highlight a significant difference among groups.