Mycophenolate Mofetil in C3 Glomerulopathy and Pathogenic Drivers of the Disease

Supplemental Material Table of Contents

Supplemental Methods:

Complement genetics and molecular studies

Genomic DNA was prepared from peripheral blood cells according to standard procedures. The entire set of complement genes (Complement gene panel 2) was analyzed in all samples by next generation sequencing (NGS) using the MiSeq next generation sequencing platform (Illumina, USA). In addition, copy number variation (CNV) analysis of the *CFH-CFHR* region was performed by Multiplex Ligation-dependent Probe Amplification (MLPA). European non-Finish population from gnomAD database was used as a control population. Variants found in coding and flanking regions were considered; synonym changes were excluded. Variants with a minor allele frequency (MAF) of 1% or lower were identified as rare variants and classified according to their pathogenicity. Only variants in the candidate genes *CFH*, *CFI*, *C3* and *CFB* or genomic rearrangements in the *CFHRs* were considered pathogenic. A variant was categorized as pathogenic if there was experimental evidence of reduced protein levels or altered function, or when at least 4 out of 6 bioinformatic predictors (SIFT, Polyphen, MutTast, MutAss, FATHMM, CADD) indicated pathogenicity. A variant was considered benign when functional data demonstrated normal protein levels and function, or 4 out of 6 predictors classified it as benign. A variant of unknown significance was considered when none of the other two criteria were met.

Serum levels of C3 and C4 were quantified using nephelometric method. Plasma levels of C5 and sC5b-9 were quantified by in-house enzyme-linked immunosorbent assays (ELISA). The detection of anti-FH antibodies and C3 nephritic factor was performed by ELISA and hemolytic assays as previously described[S1,S2], respectively.

Supplemental References:

S1. Abarrategui-Garrido C, Martínez-Barricarte R, López-Trascasa M *et al.* Characterization of complement factor H–related (CFHR) proteins in plasma reveals novel genetic variations of CFHR1 associated with atypical hemolytic uremic syndrome. *Blood* 2009; **114**: 4261–4271.

S2. Paixão-Cavalcante D, López-Trascasa M, Skattum L *et al.* Sensitive and specific assays for C3 nephritic factors clarify mechanisms underlying complement dysregulation. *Kidney Int.* 2012; **82**: 1084–1092.

Statistical analysis

Retrospective, multicenter observational cohort study. Descriptive statistics are presented as mean ± standard deviation, or median and interquartile ranges (IQR) for continuous variables, and absolute values and percentages for categorical variables.

Parametric and non-parametric tests were chosen as appropriate for descriptive comparisons of continuous variables, and chi-squared test for categorical variables. For the comparisons in smaller groups we performed a Fisher's exact test.

Cox proportional hazards regression and logistic regression models were used to analyze the main determinants of outcomes. The proportional hazard assumption was checked graphically (log–log Kaplan–Meier curves) for all covariates. These covariates in multivariable models were selected on the basis of prior knowledge and using the backwards progressive conditional elimination process.

Due to the observational nature of the study, patients were not randomly assigned to receive treatments. Thus, to minimize confounding by indication, a propensity-score matching analysis was applied to compare kidney outcomes of patients treated with or without MMF (excluding patients with conservative management), adjusting for the following potential confounding variables: baseline serum creatinine, degree of interstitial fibrosis and tubular atrophy, percentage of glomerulosclerosis and subtype of C3 glomerulopathy (C3 glomerulonephritis or dense deposit disease). A 1:1 nearest neighbor matching algorithm was applied with a caliper of 0.2 without replacement. To evaluate the quality of the different propensity-score matching models, we assessed the balance in before and after matching between the groups.

A $P<0.05$ was considered to be significant. Analyses were performed using IBM SPSS Statistics 24.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism version 7.00 (GraphPad Software, La Jolla, California, USA). The propensity-score matching was performed using SPSS R-Menu using in both cases the R statistical software version R3.1.1.

Supplemental Table 1: Baseline clinical and histologic characteristics according to age groups**.**

Variable	***Age <18 years old*** *(n=26)*	***Age >18 years old*** *(n=71)*
Baseline		
Age at diagnosis, years	9±4	39±17
Gender, males (%)	13 (50)	41 (58)
C3 glomerulonephritis/Dense deposit disease	21 / 5	60 / 11
Antecedent infection, N (%)	10 (39)	16 (23)
Clinical presentation, N (%)		
Nephrotic syndrome	12 (46)	27 (38)
Nephritic syndrome	7 (27)	22 (31)
Asymptomatic urinary abnormalities	7 (27)	22 (31)
Serum creatinine, mg/dl	0.6 [0.4–0.9]	1.9 [1.2–3.5]
CKD stages at baseline, N (%)		
1–2	21 (81)	20 (28)
3	2 (8)	19 (27)
4–5	3 (12)	32 (45)
Albumin, g/dl	2.9±0.8	3.1±0.8
Proteinuria, g/24h	2.3 [1.2–3.5]	3.1 [1.6–8]
Serum C3[a], mg/dl	42±30	67±39
Low serum C3, N (%)	21 (81)	45 (63)
Serum C4[b], mg/dl	24±10	24±9
Serum C5b–9[c], mg/l	486 [243–1260]	263 [144–763]
Elevated serum C5b–9, N (%)	24 (100)	58 (86)
Complement pathogenic variants, N (%)	4 (15)	14 (20)
Autoantibodies*, N (%)	10 (39)	19 (27)
Histopathology		
Light microscopy pattern, N (%)		
Membranoproliferative GN	21 (80)	49 (69)
Diffuse endocapillary proliferative GN	2 (8)	5 (7)
Mesangial proliferative GN	2 (8)	13 (18)
Diffuse sclerosing GN	1 (4)	4 (6)
Globally sclerotic glomeruli, %	3 [0–5]	11 [0–30]
Segmental sclerotic glomeruli, N (%)	3 (12)	13 (18)
Cellular or fibrocellular crescents, N (%)	8 (30)	16 (23)
Tubular atrophy/interstitial fibrosis, N %		
Absence	20 (77)	16 (23)
Mild	4 (15)	28 (39)
Moderate	1 (4)	18 (25)
Severe	1 (4)	9 (13)
Arterio- and arteriolosclerosis, %	0 (0)	24 (34)

* Including C3 nephritic factor and anti-factor H

Continuous variables are presented as mean±standard deviation, or median [interquartile range]

Abbreviations: eGFR: estimated glomerular filtration rate; GN: glomerulonephritis

[a] Reference values: 75–135

[b] Reference values: 14–60

[c] Reference values: <100

Supplemental Table 2: Complement pathogenic gene variants in the study patients.

Patient	Ethnicity	Age/gender	C3G/C3Nef	Genetic Variants	RefSNPs	Categorization	References for previously reported mutations
1	Caucasian	22/M	C3GN C3Nef-	*C3*: c.1656G>C; p.Trp552Cys (Het)	-	Pathogenic (RL)	Osborne et al. (2018) J Immunol. 200(7):2464-2478
2	Caucasian	19/M	DDD C3Nef-	*CFHR1:* Dup.(E2-E6) (Het)	–	Pathogenic (AF)	Tortajada et al. (2013) J Clin Invest. 123(6).
3	Caucasian	21/M	C3GN C3Nef-	*C3*: c.1379T>G; p.Val460Gly (Het)	-	Pathogenic (RL)	Not previously described
4	Caucasian	47/M	C3GN C3Nef-	*CFI*: c.1234G>A; p.Val412Met (Het)	rs371432629	Pathogenic (RL)	Pras et al. (2015) J Med Genet. 52(7):484-92. Osborne et al. (2018) J Immunol. 200(7):2464-2478
5	Caucasian	9/M	C3GN C3Nef-	*C3:* c.1269+1G>A (Het)	-	Pathogenic (RL)	Osborne et al. (2018) J Immunol. 200(7):2464-2478
6	Caucasian	4/M	C3GN C3Nef-	*C3:* c.2770G>A; p.Gly924Ser (Het)	-	Pathogenic (RL)	Osborne et al. (2018) J Immunol. 200(7):2464-2478
7	Caucasian	23/F	C3GN C3Nef-	*C3:* c.2203C>T; p.Arg735Trp (Het)	rs117793540	Pathogenic (RL)	Frémeaux-Bacchi et al. (2008) Blood, 112(13):4948-4952 Osborne et al. (2018) J Immunol. 200(7):2464-2478
8	Caucasian	28/M	C3GN C3Nef-	*CFI:* c.1071T>G; p.Ile357Met (Het)	rs200881135	Pathogenic (RL)	Westra (2010) Nephrol Dial Transplant 25, 2195-2202 Osborne et al. (2018) J Immunol. 200(7):2464-2478
9	Caucasian	57/M	DDD C3Nef-	*CFH:* c.328G>T; p. Ala110Ser (Hom)	-	Pathogenic (RL)	Martin-Merinero et al. (2018) Kidney International.
10	Caucasian	20/M	C3GN C3Nef-	*CFHR1:* Dup.(PROM-E3) (Het)	-	Pathogenic (AF)	Not previously described
11	Caucasian	26/M	DDD C3Nef-	*C3:* c.1898A>G; Lys633Arg (Het)	rs140655115	Pathogenic (RL)	Moore I, et. al. (2010) Blood. 115(2):379-87 Iatropoulus et al. (2016) Mol Immunol.71:131-142. Osborne et al. (2018) J Immunol. 200(7):2464-2478
12	Caucasian	22/F	C3GN C3Nef-	*C3:* c.4339T>C; p.Tyr1447His (Het)	-	Pathogenic (RL)	Not previously described
13	Caucasian	26/M	C3GN C3Nef-	*CFH:* c.328G>T; p.Ala110Ser (Het)	-	Pathogenic (RL)	Martin-Merinero et al. (2018) Kidney International
14	Caucasian	53/M	C3GN C3Nef+	*CFH:* c.1132G>T; p.Gly378* (Het)	-	Pathogenic (RL)	Not previously described
15	Caucasian	12/M	C3GN C3Nef-	*CFHR3::CFHR1* hybrid gene (Het)	-	Pathogenic (AF)	Not previously described
16	Caucasian	34/M	C3GN C3Nef-	*C3:* c.3481C>A; p.Gln1161Lys (Het)	-	Pathogenic (AF)	Frémeaux-Bacchi et al. (2008) Blood, 112(13):4948-4952 Schramm (2015) Blood 9; 125(15): 2359–2369. Osborne et al. (2018) J Immunol. 200(7):2464-2478
17	Caucasian	53/M	C3GN C3Nef+	*CFI:* c.1508_1510del; p.Phe503del (Het)	-	Pathogenic (RL)	Not previously described
18	Caucasian	24/M	C3GN C3Nef-	*CFB:* c.724A>C; p.Ile242Leu (Het)	rs144812066	Pathogenic (AF)	Marinozzi et al. (2014) J. Am. Soc Nephrol. 25(9)

Abbreviations: C3G: C3 glomerulopathy; C3Nef: C3 nephritic factor; F: female; Het: heterozygote; Hom: homozygote; M: male.
Pathogenicity is indicated and the consequences of the mutations depicted as follows: RL, Reduced Levels; AF, Altered Function

Supplemental Table 3: Clinical characteristics according to the presence of complement genetic variants and/or autoantibodies against complement components*.

Variable	***Pathogenic variants*** *(n=18)*	***Variants of unknown significance*** *(n=42)*	***No complement abnormalities*** *(n=37)*	***P***	***Presence of autoantibodies**** *(n=29)*	***Absence of autoantibodies*** *(n=68)*	***P***
Age at diagnosis, years	26±15	36±22	30±20	0.17	31±21	32±20	0.84
<18 years % / >18 years %	4 / 14	9 / 33	13 / 24	0.34	10 / 19	16 / 52	0.26
Gender, males (%)	16 (89)	21 (50)	17 (46)	0.007	13 (45)	41 (60)	0.16
C3 glomerulonephritis /Dense deposit disease	15(83) / 3(17)	36(86) / 6(14)	30(81) / 7(19)	0.75	20(69) / 9(31)	61(90) / 7(10)	0.01
Antecedent infection, N (%)	6 (33)	9 (21)	11 (30)	0.56	13 (45)	41 (60)	0.16
Clinical presentation, N (%)				0.88			0.66
Nephrotic syndrome	7 (39)	19 (45)	13 (35)		12 (41)	27 (40)	
Nephritic syndrome	5 (28)	11 (26)	13 (35)		7 (24)	22 (32)	
Asymptomatic urinary abnormalities	6 (33)	12 (29)	11 (30)		10 (35)	19 (28)	
Serum creatinine, mg/dl	1.3 [0.8–2.9]	1.5 [0.8–3]	1.8 [0.7–3.3]	0.78	1.2 [0.8–2.5]	1.5 [0.7–3.2]	0.48
eGFR at diagnosis, ml/min/1,73m^2	59 [24–119]	56 [18–117]	39 [17–129]	0.89	59 [24–137]	50 [16–117]	0.37
Albumin, g/dl	3.2±0.8	3±0.8	3.1±0.8	0.62	3±0.9	3.1±0.8	0.85
Proteinuria, g/24h	3 [1.2–7.7]	3 [1.8–5]	3 [1.7–9]	0.68	3.4 [2.4–9.7]	2.7 [1.5–5.3]	0.10
Serum C3[a], mg/dl	47±30	67±41	63±43	0.25	49±38	66±40	0.04
Serum C5b–9[b], mg/l	430 [220-843]	213 [135-615]	370 [211-1060]	0.14	618 [205-1042]	260 [155-620]	0.02
Concomitant presence of autoantibodies**, N (%)	2 (11)	12 (29)	15 (41)	0.08	–	–	–

* Information regarding complement pathogenic abnormalities is provided in Supplementary Table S2

** Including C3 nephritic factor and anti-factor H.

Continuous variables are presented as mean±standard deviation, or median [interquartile range]

Abbreviations: eGFR: estimated glomerular filtration rate

[a] Reference values: 75–135

[b] Reference values:<100

Supplemental Table 4: Clinical characteristics of patients who achieved or not remission, according to baseline eGFR (classified into three groups: <30; 31-59; >60 ml/min/1.73m^2)

Variable	***Remission (complete + partial)***			***P***	***No remission***			***P***
	Baseline eGFR >60 (n=25)	*eGFR 31–59 (n=11)*	*eGFR <30 (n=10)*		*Baseline eGFR >60 (n=16)*	*eGFR 31–59 (n=10)*	*eGFR <30 (n=25)*	
Age at diagnosis, years	18±12	34±16	39±27	0.003	19±13	40±15	47±20	<0.001

Adult % / Pediatric %	44 / 56	91 / 9	70 / 30	0.06	56 / 44	90 / 10	100 / 0	<0.001
Gender, males (%)	9 (36)	6 (55)	6 (60)	0.35	7 (44)	8 (80)	18 (72)	0.09
C3 glomerulonephritis /Dense deposit disease	22 / 3	10 / 1	10 / 0	0.28	10 / 6	8 / 2	21 / 4	0.13
Antecedent infection, N (%)	5 (20)	0 (0)	5 (50)	0.15	4 (25)	3 (30)	9 (36)	0.76
Clinical presentation, N (%)				0.55				0.77
Nephrotic syndrome	13 (52)	3 (27)	1 (10)		10 (63)	4 (40)	8 (32)	
Nephritic syndrome	4 (16)	3 (27)	7 (70)		1 (6)	2 (20)	12 (48)	
Asymptomatic urinary abnormalities	8 (32)	5 (46)	2 (20)		5 (31)	4 (40)	5 (20)	
Serum creatinine, mg/dl	0.7 [0.5–0.8]	1.4 [1.3–1.9]	4.2 [2.7–5.8]	<0.001	0.8 [0.5–1]	1.6 [1.3–1.9]	3.5 [3–6.6]	<0.001
Albumin, g/dl	3.2±0.9	3.2±0.8	3.2±0.8	0.97	2.8±0.8	2.9±0.7	3.1±0. 8	0.51
Proteinuria, g/24h	2.5 [1.3–3.2]	4.1 [1.2–7.9]	2.4 [1.6–3.3]	0.32	4.5 [2.8–5.9]	4.5 [1.3-10]	4.1 [1.7–9]	0.68
Serum C3[a], mg/dl	41±31	66±50	73±40	0.05	58±42	69±45	72±34	0.52
Serum C5b–9[b], mg/l	441 [177-1129]	329 [125-587]	303 [191-719]	0.58	475 [290-1036]	843 [281-1331]	191[124-513]	0.006
Complement pathogenic variants*, N (%)	3 (12)	3 (27)	0 (0)	0.34	5 (31)	3 (30)	4 (33)	0.74
Autoantibodies**, N (%)	9 (36)	2 (18)	3 (30)	0.56	4 (25)	4 (40)	7 (28)	0.70
Histopathology								
Light microscopy pattern, N (%)				0.003				0.27
Membranoproliferative GN	19 (76)	7 (64)	4 (40)		14 (88)	6 (60)	15 (60)	
Diffuse endocapillary proliferative GN	3 (12)	2 (18)	0 (0)		0 (0)	0 (0)	2 (8)	
Mesangial proliferative GN	2 (8)	2 (18)	3 (30)		1 (6)	2 (20)	2 (8)	
Diffuse sclerosing GN	1 (4)	0 (0)	3 (30)		1 (6)	2 (2)	1 (4)	
Globally sclerotic glomeruli, %	0 [0–5]	5 [0–23]	8 [0–28]	0.03	2.6 [0–19]	7.2 [0–20]	31 [20-59]	<0.001
Cellular or fibrocellular crescents, N (%)	3 (12)	2 (18)	6 (60)	0.01	3 (19)	3 (30)	7 (28)	0.54
Tubular atrophy/interstitial fibrosis, N %								<0.001
Absence	14 (56)	4 (36)	4 (40)		10 (63)	2 (20)	2 (8)	
Mild	9 (36)	5 (46)	5 (50)		3 (19)	5 (50)	5 (20)	
Moderate	1 (4)	2 (18)	0 (0)		2 (13)	2 (20)	12 (48)	
Severe	1 (4)	0 (0)	1 (10)		1 (6)	1 (10)	6 (24)	
Treatment				0.43				0.07
No immunosuppression, N (%)	3 (12)	0 (0)	0 (0)		1 (6)	1 (10)	12 (48)	
Corticosteroids + MMF, N (%)	15 (60)	10 (91)	8 (80)		4 (25)	3 (30)	2 (8)	
Other immunosuppression, N (%)	6 (24)	0 (0)	1 (10)		9 (56)	5 (50)	8 (32)	
Eculizumab, N (%)	1 (33)	1 (9)	1 (10)		2 (13)	1 (10)	3 (12)	

* Information regarding complement abnormalities is provided in Supplementary Table S2

** Including C3 nephritic factor and anti-factor H.

Continuous variables are presented as mean±standard deviation, or median [interquartile range]

[a] Reference values: 75–135

[b] Reference values:<100

Supplemental Table 5: Multivariable logistic regression analysis for predictors of remission of the disease*

	Univariable		*Multivariable*	
Variable	***Odds ratio (95% CI)***	***P***	***Odds ratio (95% CI)***	***P***
Gender (male vs female)	**0.16 (0.25–1.00)**	**0.05**	**0.18 (0.53–0.61)**	**0.006**
Age (adult vs pediatric)	**0.21 (0.03–1.30)**	**0.09**	**0.21 (0.05–0.82)**	**0.02**
Dense deposit disease/ C3 glomerulonephritis	0.23 (0.03–1.64)	0.14		
CKD stages at baseline				
3 versus 1–2	1.19 (0.19–7.38)	0.84		
4–5 versus 1–2	0.56 (0.07–4.72)	0.59		
24-hour proteinuria (>3.5g/24h vs <3.5 g/24h)	**0.12 (0.02–0.54)**	**0.006**	**0.15 (0.04–0.51)**	**0.003**
Serum C3 levels (high vs low)	4.67 (0.91–12.3)	0.06		
Complement pathogenic variants (yes vs no)	0.43 (0.14–2.75)	0.42		
Autoantibodies against complement regulators (yes vs no)	1.16 (0.24–5.62)	0.85		
Glomerulosclerosis				
25–50% vs <25%	1.38 (0.14–3.76)	0.67		
50–75% vs <25%	3.2 (0.26–8.22)	0.23		
>75% vs <25%	0.4 (0.07–2.72)	0.39		
Interstitial fibrosis/tubular atrophy				
25–50% vs <25%	3.11 (0.49–8.64)	0.23		
50–75% vs <25%	0.58 (0.05–6.82)	0.66		
>75% vs <25%	0.41 (0.02–7.11)	0.54		
Treatment with MMF plus corticosteroids (yes vs no)	**3.36 (2.48–4.68)**	**0.001**	**5.72 (4.48–7.08)**	**0.001**

*Number of events: 46

Abbreviations: CI: confidence interval; CKD: chronic kidney disease; MMF: mycophenolate mofetil; vs: versus

Supplemental Table 6: Clinical characteristics according to the development of kidney failure.

Variable	*Kidney failure*	*No kidney failure*	*p*
Baseline			
Patients, N (%)	40 (41)	57 (59)	
Age at diagnosis, years	36±19	28±21	0.06
Adult % / Pediatric %	88 / 12	63 / 37	0.008
Gender, males (%)	27 (68)	27 (47)	0.04
C3 glomerulonephritis / Dense deposit disease, N	30 / 10	51 / 6	0.06
Antecedent infection, N (%)	14 (35)	12 (21)	0.12
Clinical presentation, N (%)			0.63
Nephrotic syndrome	16 (40)	23 (40)	
Nephritic syndrome	13 (32)	16 (28)	
Asymptomatic urinary abnormalities	11 (28)	18 (32)	
Serum creatinine, mg/dl	2.8 [1.2–4.3]	1 [0.7–2]	<0.001
eGFR at diagnosis, ml/min/1,73m^2	24 [11–60]	100 [34–114]	<0.001
Albumin, g/dl	3±0.8	3.1±0.8	0.31
Proteinuria, g/24h	4.4 [1.8–9]	2.7 [1.2–4.2]	0.02
Serum C3[a], mg/dl	64±35	59±43	0.53
Complement pathogenic variants, N (%)	9 (23)	9 (16)	0.40
Autoantibodies*, N (%)	15 (38)	16 (28)	0.33
Serum C5b–9[b], mg/l	298 (144-770)	394 (188-892)	0.25
Histopathology			
Light microscopy pattern, N (%)			0.54
Membranoproliferative GN	29 (73)	41 (72)	
Diffuse endocapillary proliferative GN	1 (2)	6 (11)	
Mesangial proliferative GN	8 (20)	7 (12)	
Diffuse sclerosing GN	2 (5)	3 (5)	
Globally sclerotic glomeruli, %	22 [6–40]	4 [0–8]	<0.001
Cellular or fibrocellular crescents, N (%)	10 (25)	14 (25)	0.96
Tubular atrophy/interstitial fibrosis, N %			<0.001
Absence	10 (25)	26 (46)	
Mild	8 (20)	24 (42)	
Moderate	14 (35)	5 (9)	
Severe	8 (20)	2 (4)	
Arterio- and arteriolosclerosis, %	13 (33)	11 (19)	0.14
Treatment and Outcomes			<0.001
No immunosuppression, N (%)	11 (28)	6 (11)	
Corticosteroids + MMF, N (%)	6 (15)	36 (63)	
Other immunosuppression, N (%)	17 (42)	12 (21)	
Eculizumab, N (%)	6 (15)	3 (5)	
Remission (complete+partial), N (%)	0 (0)	46 (81)	<0.001

* Including C3 nephritic factor and anti-factor H

Continuous variables are presented as mean±standard deviation, or median [interquartile range]
Abbreviations: eGFR: estimated glomerular filtration rate; GN: glomerulonephritis; MMF: mycophenolate mofetil

[a] Reference values: 75–135
[b] Reference values: <100

Supplemental Table 7: Cox proportional hazard regression analysis for association between covariables and kidney failure*.

	Univariable		*Multivariable*	
Variable	***Hazard ratio (95% CI)***	***P***	***Hazard ratio (95% CI)***	***P***
Gender (male vs female)	2.94 (1.01–8.53)	0.05		
Age (adult vs pediatric)	1.18 (0.23–6.03)	0.84		
Antecedent infection (yes vs no)	1.47 (0.59–3.65)	0.41		
Dense deposit disease/C3 glomerulonephritis	2.45 (0.77–7.85)	0.13		
CKD stages at baseline				
3 versus 1–2	**2.81 (0.84–9.32)**	**0.09**	**3.51 (1.24–6.96)**	**0.02**
4–5 versus 1–2	**5.45 (1.39–8.43)**	**0.02**	**4.14 (1.43–9.63)**	**0.009**
Serum C3 levels (low vs high)	1.01 (0.36–2.79)	0.98		
24-hour proteinuria (>3.5g/24h vs <3.5 g/24h)	2.58 (1.12–5.95)	0.04		

Complement pathogenic variants (yes vs no)	1.50 (0.73–3.78)	0.39		
Autoantibodies against complement regulators (yes vs no)	1.33 (0.48–3.71)	0.58		
Glomerulosclerosis				
25–50% vs <25%	1.18 (0.32–4.39)	0.81		
50–75% vs <25%	1.13 (0.29–4.45)	0.86		
>75% vs <25%	1.08 (0.08–9.76)	0.95		
Interstitial fibrosis/tubular atrophy				
25–50% vs <25%	1.34 (0.33–5.42)	0.68		
50–75% vs <25%	2.83 (0.62–6.81)	0.18		
>75% vs <25%	**5.77 (1.03–13.1)**	**0.04**	**6.45 (1.88–9.52)**	**0.004**
RAS blockade (yes vs no)	1.03 (0.61–1.73)	0.92		
Corticosteroids + MMF (yes vs no)	**0.27 (0.09–0.84)**	**0.02**	**0.25 (0.09–0.62)**	**0.003**
Other immunosuppressive therapy (yes vs no)	0.64 (0.18–2.27)	0.49		
Treatment with eculizumab (yes vs no)	0.37 (0.09–0.84)	0.18		

*Number of events: 40

Abbreviations: CI: confidence interval; MMF: mycophenolate mofetil; RAS: renin-angiotensin system; vs: versus

Supplemental Table 8: Clinical and histopathologic characteristics of patients before and after propensity score matching analysis

Variable	*Before propensity-score matching*				*After propensity-score matching*			
	MMF (n=42)	Non-MMF (n=55)	p	d*	MMF (n=34)	Non-MMF (n=34)	p	d*
Baseline								
Age at diagnosis, years	30±19	33±21	0.56		29±20	30± 22	0.75	
Gender, males (%)	23 (55)	31 (56)	0.88		17 (50)	19 (56)	0.63	
C3 glomerulonephritis / Dense deposit disease, N	40 / 2	41 / 14	<0.001	-0.96	30 / 4	29 / 5	0.80	0.17
Serum creatinine, mg/dl	1.3 [0.7-2.4]	1.8 [0.8-3.5]	0.05	-0.67	1 [0.6-2.4]	1.1 [0.7-2.1]	0.75	0.07
Albumin, g/dl	3.1±0.8	3±0.8	0.65		3.2±0.7	3.1±0.8	0 .59	
Serum C3[a], mg/dl	61±45	61±36	0.96		62±46	68±38	0.56	
Serum C4[b], mg/dl	23±8	25±10	0.29		22±9	25±11	0.26	
Proteinuria, g/24h	3 [1.8-4.6]	2.9 [1.5-8.8]	0.69	-0.31	2.6 [1.5-4]	2.3 [1-6]	0.81	-0.19
Histopathology								
Light microscopy pattern, N (%)			0.26				0.59	
Membranoproliferative GN	31 (74)	39 (71)			25 (74)	25 (74)		
Endocapillary proliferative GN	4 (10)	3 (6)			2 (6)	4 (12)		
Mesangial proliferative GN	6 (14)	9 (16)			2 (6)	5 (14)		
Diffuse sclerosing GN	1 (2)	4 (7)			5 (14)	0 (0)		
Globally sclerotic glomeruli, %	5 [0–9]	20 [0–40]	0.01	-0.61	4 [0–11]	4 [0–21]	0.66	-0.14
Cellular or fibrocellular crescents, N (%)	11 (26)	13 (24)	0.77		8 (24)	6 (18)	0.55	
Tubular atrophy/interstitial fibrosis, N %			0.09	-0.44			0.78	0.04
Absence	18 (43)	18 (33)			16 (47)	13 (38)		
Mild	17 (40)	15 (27)			11 (32)	13 (38)		
Moderate	4 (10)	15 (27)			4 (12)	7 (21)		
Severe	3 (7)	7 (13)			3 (9)	1 (3)		
Arterio- and arteriolosclerosis, %	7 (17)	17 (31)	0.11		5 (15)	9 (26)	0.23	
Outcomes								
Follow-up, months	49 [23–97]	45 [12–81]	0.27		38 [16–90]	43 [19–50]	0.72	
Complete remission, N (%)	15 (36)	3 (6)	<0.001		13 (38)	1 (3)	<0.001	
Remission (complete+partial), N (%)	33 (79)	13 (24)	<0.001		28 (82)	9 (26)	<0.001	
Kidney failure, N (%)	6 (14)	34 (62)	<0.001		4 (12)	18 (53)	<0.001	

*d: Cohen's standardized mean differences

Continuous variables are presented as mean±standard deviation, or median [interquartile range]
Abbreviations: GN: glomerulonephritis; MMF: mycophenolate mofetil;.

[a] Reference values: 75–135
[b] Reference values: 14–60

Supplemental Table 9: Cox proportional hazard regression analysis for predictors of no response to corticosteroids plus mycophenolate mofetil*

	Univariable		*Multivariable*	
Variable	***Hazard ratio (95% CI)***	***P***	***Hazard ratio (95% CI)***	***P***
Gender (male vs female)	1.16 (0.63–2.15)	0.64		
Age (adult vs pediatric)	1.07 (0.96–1.16)	0.06		
Dense deposit disease/C3 glomerulonephritis	0.28 (0.02–5.11)	0.39		
CKD stages at baseline				
1–2 versus 3	0.48 (0.07–3.38)	0.46		
1–2 versus 4–5	0.73 (0.21–4.53)	0.53		
24-hour proteinuria (>3.5g/24h vs <3.5 g/24h)	**1.48 (1.21–1.96)**	**0.06**	**1.23 (1.02–1.46)**	**0.03**
Complement pathogenic variants (no vs yes)	0.99 (0.09–5.54)	0.99		
Autoantibodies against complement regulators (no vs yes)	0.19 (0.02–1.61)	0.13		
Glomerulosclerosis				
25–50% vs <25%	1.08 (0.47–2.47)	0.85		
50–75% vs <25%	2.25 (0.86–5.92)	0.12		
>75% vs <25%	5.21 (0.58–7.32)	0.21		
Interstitial fibrosis/tubular atrophy				
25–50% vs <25%	1.01 (0.43–2.36)	0.98		
50–75% vs <25%	2.04 (0.87–4.82)	0.12		
>75% vs <25%	1.66 (0.66–4.22)	0.28		

*Number of events: 9

Abbreviations: CI: confidence interval; vs: versus

Supplemental Table 10: Clinical, histopathologic and genetic characteristics of patients treated with mycophenolate mofetil plus corticosteroids according to relapse of the disease.

Variable	***Relapse of the disease*** *(n=11)*	***No relapse after initial remission*** *(n=22)*	***p***
Baseline			
Age at diagnosis, years	20±17	35±20	0.03
Gender, males (%)	5 (46)	11 (50)	0.81
Adult % / Pediatric %	36 / 64	82 / 18	0.009
C3 glomerulonephritis / Dense deposit disease, N	11 / 0	21 / 1	0.48
Clinical presentation, N (%)			0.16
Nephrotic syndrome	6 (55)	5 (23)	
Nephritic syndrome	3 (27)	8 (36)	
Asymptomatic urinary abnormalities	2 (18)	9 (41)	
Serum creatinine, mg/dl	0.6 [0.5–1.3]	1.8 [0.8–2.9]	<0.001
Albumin, g/dl	3.1±0.6	3.3±0.9	0.56
Proteinuria, g/24h	1.9 [1.1–3.4]	2.9 [1.8–4]	0.26
Serum C3[a], mg/dl	42±36	69±44	0.08
Serum C5b–9[b], mg/l	600 [220–1549]	295 [182–621]	0.12
Histopathology			
Globally sclerotic glomeruli, %	0 [0–6]	5 [0–11]	0.19
Tubular atrophy/interstitial fibrosis, N %			0.14
Absence	6 (55)	8 (36)	
Mild	5 (46)	10 (46)	
Moderate	0 (0)	2 (9)	
Severe	0 (0)	2 (9)	
Complement genetic analysis			
Complement abnormalities[c], N (%)			0.41
None	5 (46)	10 (46)	
Variants of unknown significance	4 (36)	11 (50)	
Pathogenic variants	2 (18)	1 (4)	
Autoantibodies, N (%)	1 (9)	7 (32)	0.15
Treatment			
Mean initial daily dose of corticosteroids, mg/kg/day	0.9±0.6	0.9±0.3	0.94
Median initial duration of corticosteroids, months	7 [4–20]	13 [6–18]	0.12
Mean initial daily dose of MMF, mg/day	1110±720	1000±500	0.75
Median initial duration of MMF, months	7 [2–17]	11 [7–27]	0.11
Serum creatinine at remission[d], mg/dl	0.7 [0.5–0.8]	0.9 [0.7–1.5]	0.09
Serum C3 at remission[d], mg/dl	66±47	81±29	0.44
Serum albumin at remission[d], g/dl	3.8±0.7	3.9±0.3	0.55
Proteinuria at remission[d], g/24h	0.7 [0.2–1.6]	0.9 [0.4–1.5]	0.76

Continuous variables are presented as mean±standard deviation, or median [interquartile range]
Abbreviations: MMF: mycophenolate mofetil

[a] Reference values: 75–135
[b] Reference values: <100
[c] Information regarding complement pathogenic abnormalities is provided in Supplementary Table S2
[d] Including both partial+complete remission

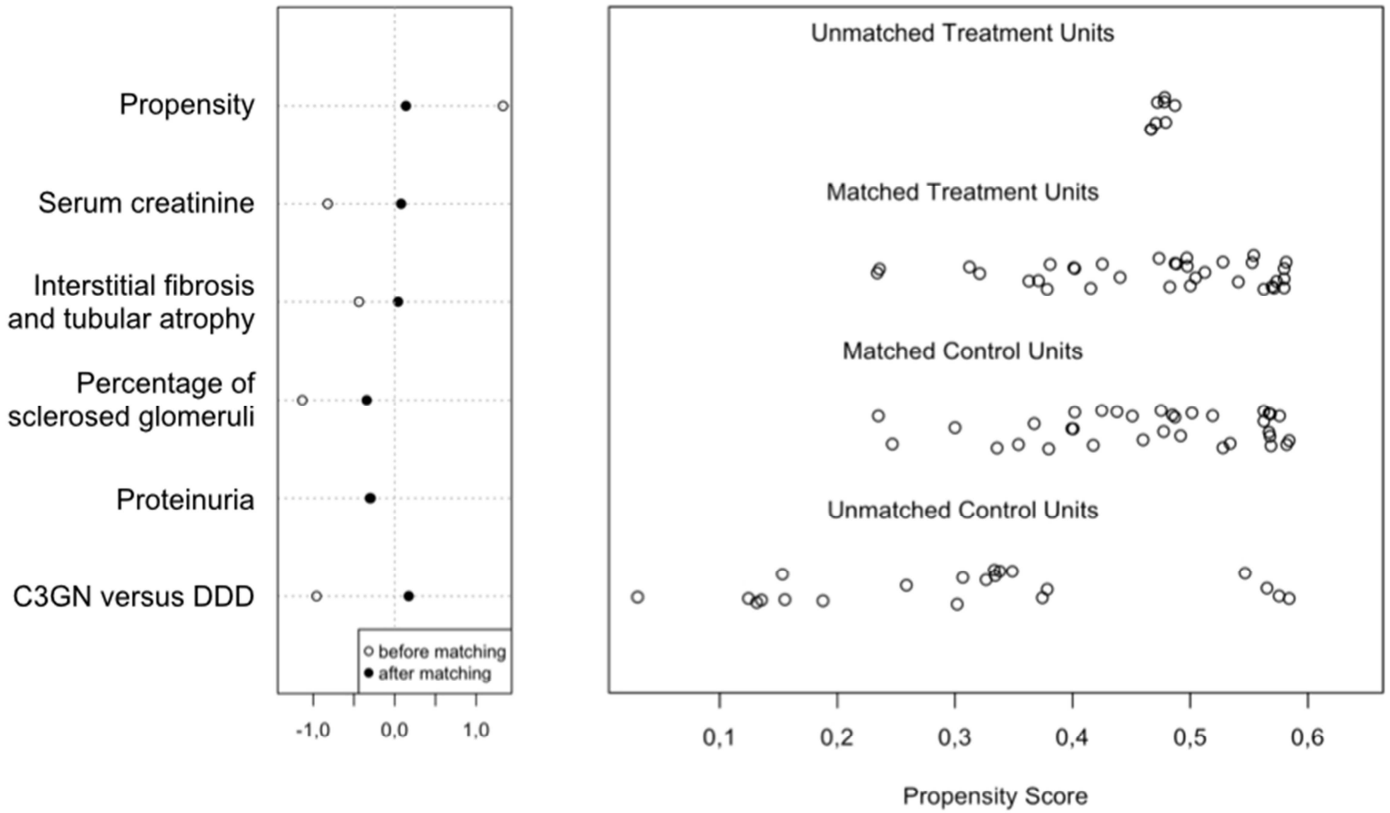
Supplemental Table 11: Cox proportional hazard regression analysis for predictors of relapse in patients treated with corticosteroids plus mycophenolate mofetil*

	Univariable		*Multivariable*	
Variable	***Hazard ratio (95% CI)***	***P***	***Hazard ratio (95% CI)***	***P***
Gender (male vs female)	1.61 (0.22–2.18)	0.64		
Age (adult vs pediatric)	0.63 (0.83–4.90)	0.66		
C3 glomerulonephritis/Dense deposit disease	0.98 (0.75–1.22)	0.72		
CKD stages at remission				
3 versus 1–2	1.29 (0.28–5.94)	0.74		
4–5 versus 1–2	3.52 (0.53–7.58)	0.62		
24-hour proteinuria at remission (>3.5g/24h vs <3.5 g/24h)	1.13 (0.79–1.62)	0.51		
Median length of corticosteroids (>6 months vs <6 months)	0.98 (0.87–1.09)	0.68		
Median length of MMF (>12 months vs <12 months)	**0.27 (0.03–0.74)**	**0.02**	**0.18 (0.04–0.87)**	**0.03**

*Number of events: 11

Abbreviations: CI: confidence interval; MMF: mycophenolate mofetil

Supplemental Figure 1: Dot plot for the propensity scores of patients in the MMF and non-MMF groups showing individual units in the dataset and whether they were matched or not.



Supplemental Figure 2: Kaplan-Meier curves for kidney survival according to cluster analysis in patients treated with corticosteroids plus MMF versus other regimens.