Pediatric Nephrology (2020) 35:153–162
https://doi.org/10.1007/s00467-019-04388-3

ORIGINAL ARTICLE

Clinicopathological features of C3 glomerulopathy in children: a single-center experience

Keri A. Drake[1] · Natalie Ellington[2] · Jyothsna Gattineni[1] · Jose R. Torrealba[2] · Allen R. Hendricks[3]

Received: 22 January 2019 / Revised: 4 September 2019 / Accepted: 27 September 2019 / Published online: 30 October 2019
© IPNA 2019

Abstract

Background C3 glomerulopathy (C3G) is defined by dominant glomerular deposition of C3 and minimal or no immunoglobulin, with two subtypes—dense deposit disease (DDD) and C3 glomerulonephritis (C3GN)—distinguished by features on electron microscopy (EM). Given that this rare disease has generally unfavorable yet highly variable outcomes, we sought out to review the histopathology, complement/genetic studies, and renal outcomes of pediatric patients with C3G at our institution.
Methods All native kidney biopsies performed in a single pediatric hospital over a 10-year period were reviewed for features of C3G. Of 589 biopsy reports, we identified 9 patients fulfilling the diagnostic criteria for C3G and retrospectively reviewed their clinical chart and renal biopsy findings.
Results We identified 4 patients with DDD, 4 with C3GN, and 1 indeterminate case, with features of both C3GN and DDD. Five patients were positive for one or more nephritic factors (C3NeF, C4NeF, C5NeF) with 1 patient additionally positive for complement factor H (CFH) autoantibody. Genetic testing done in 5 of the 9 patients failed to identify any causative mutations. Three patients showed progressive renal dysfunction over a mean follow-up period of 33 months.
Conclusions Complement and genetic studies are now routinely recommended for patients with a histopathological diagnosis of C3G. Careful interpretation of these studies and their prognostic and therapeutic implications in conjunction with biopsy findings is needed to further understand the pathophysiology of this rare disease in children.

Keywords C3 glomerulopathy · dense deposit disease · C3 glomerulonephritis · nephritic factor · children

Introduction

Though defined by its characteristic histology, C3 glomerulopathy (C3G) has emerged as a disease process primarily driven by abnormal complement activation, deposition, and/or degradation. Renal biopsy findings demonstrate predominant glomerular C3 fragment deposition, with C3 immunofluorescence (IF) at least two orders of magnitude brighter than any other immune reactant and electron-dense deposits on EM [1, 2]. Dense deposit disease (DDD) and C3 glomerulonephritis (C3GN) are subsets of C3G, both of which are defined by dominant C3 IF staining. While heterogenous histologic changes are seen by light microscopy, distinction between the two entities is made by EM [3]. In DDD, there is hyperosmiophilic, electron-dense transformation of the lamina densa of the glomerular basement membrane. In C3GN, mesangial and/or glomerular basement membrane deposits are present in variable combinations of subepithelial, subendothelial and/or less dense, discontinuous intramembranous deposits [4, 5]. The pathogenesis of C3G includes dysregulation of the complement alternative pathway (AP). Identification of genetic mutations, circulating autoantibodies, and/or abnormal complement profiles is somewhat heterogenous, and the etiology has generally been considered to be unknown in most patients [6].

C3G is considered an ultra-rare disease, with an estimated incidence of 1–2 per million per total population and is

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00467-019-04388-3) contains supplementary material, which is available to authorized users.

✉ Allen R. Hendricks
allen.hendricks@UTSouthwestern.edu

[1] Division of Pediatric Nephrology, UT Southwestern Medical Center, 1935 Medical District Drive, Dallas, TX 75235, USA

[2] Department of Pathology, UT Southwestern Medical Center, 6210 Harry Hines Blvd, Dallas, TX 75390-9234, USA

[3] Department of Pathology, UT Southwestern Medical Center, 5959 Harry Hines Blvd., Suite HP3-378, Dallas, TX 75390-9234, USA

responsible for 3–5% of all pediatric nephrotic syndromes [7–9]. Histological recurrence post-transplant occurs in C3G and DDD and has been reported as possibly universal in DDD [3], which can lead to graft loss in a high percentage of patients making it a potentially devastating disease for children. Previous publications describing C3G have largely incorporated children and adults together, with limited pediatric centered studies detailing serologic and genetic complement data [8, 10–15]. For instance, Servais et al. reported that 17 out of 29 DDD patients and 14 out of 56 C3GN patients in their study were children and found progression to end-stage kidney disease (ESKD) and low serum C3 were reportedly more common in DDD compared to C3GN [16]. Recent research by Bomback et al. involving pediatric and adult patients found no differences between DDD and C3GN in progression to CKD and low C3 at diagnosis, with 3 out of 24 DDD patients and 32 out of 87 C3GN patients in their study < 18 years old [17]. These studies both included autoantibody and genetic testing and consistently identified complement regulatory genes and acquired autoantibodies in patients with C3G, including C3NeF, which stabilizes the AP C3 convertase, preventing its inactivation. However, there have been considerable advances in complement pathway assays, with increased numbers of autoantibodies and pathogenic mutations identified. Since standardized serologic and genetic testing for inherited and acquired abnormalities of AP of complement has only recently become common practice, with limited data in pediatric C3G patients, we sought to retrospectively review this information along with a comprehensive histopathological analysis for all cases of pediatric C3G at our single institution over a 10-year period. Our inclusion of available complement studies enhances identification of underlying abnormalities, which is necessary in larger studies to contribute to guiding treatment, predicting outcomes, and facilitating development of complement-directed therapeutics.

Materials and methods

Patient selection

Using the natural language processing tool in Cerner electronic medical record software a search was conducted to retrieve renal biopsies accessioned at Children's Health Dallas from April 2007 through February 2017. The following terms were used for the search: membranoproliferative glomerulonephritis, membranoproliferative glomerulonephritis with dominant C3, dense deposit disease, acute diffuse proliferative glomerulonephritis, diffuse proliferative glomerulonephritis, endocapillary proliferative glomerulonephritis, post-streptococcal glomerulonephritis, and post-infectious glomerulonephritis. All cases with one or more of these terms in the original final diagnosis, and C3 dominant staining (defined as ≥ 2 orders of magnitude more than any other immune reactant on a semiquantitative scale of 0–4) by routine IF evaluation were included for use in the study. All retrieved cases were examined by two pathologists (N.E. and A.R.H.) to confirm that they met inclusion criteria, thus satisfying pathologic criteria for a diagnosis of C3G. The study was approved by the UT Southwestern Institutional Review Board having met all applicable requirements.

Biopsy sample processing techniques

All cases were processed by light, IF, and EM using standard techniques [18]. Kidney biopsy samples were fixed in buffered formalin, dehydrated in graded alcohols, and embedded in paraffin using standard methods. Serial 3-mm-thick sections were cut and treated with hematoxylin and eosin (H and E), Jones methenamine silver, Masson trichrome, and periodic acid–Schiff (PAS) reagent.

Samples submitted for IF studies were transported in Michel's or other acceptable transport medium, washed in buffer and frozen in a cryostat. Sections, cut at 4 μm, were rinsed in buffer and reacted with fluorescein-tagged polyclonal rabbit anti-human antibodies to immunoglobulin (Ig) G, IgA, IgM, C3, C1q, albumin and fibrinogen [Agilent (formerly Dako), Carpenteria, CA] for 1 h, rinsed and a coverslip applied using aqueous mounting media. The staining intensity was graded 0 to 4+ on a semiquantitative scale.

EM was performed on specimens submitted for ultrastructural examination. The specimen was dehydrated using graded alcohols, and embedded in epon resin. Sections 1 mm thick were cut using an ultramicrotome, stained with toluidine blue and examined with a light microscope. Thin sections were examined in a Hitachi H-7500 transmission electron microscope. Photomicrographs were routinely taken at varying magnifications.

Clinical data collection

Clinical and laboratory data at the time of the first renal biopsy were manually obtained through retrospective chart review and included age, sex, ethnicity/race, presenting symptoms, family history of renal disease, serum creatinine, serum albumin, serum C3 and C4, and serum antibodies (anti-streptolysin-O titer ASOT, ANA, ANCA, dsDNA). Hematuria was defined by urine dipstick analysis and/or > 4 RBCs/hpf. Proteinuria was defined by the protein-creatinine ratio (g/g) in spot urine > 0.2. Estimated glomerular filtration rate (GFR) was determined using the revised Schwartz equation. When performed, complement autoantibodies, assays for complement biomarkers, and genetic screening results were obtained, with performing laboratories, detailed methods and established reference ranges for each test detailed in Table 3 and in supplementary material.

Table 1 Clinical and laboratory findings in patients with C3 glomerulopathy

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Age at diagnosis	8 years	11 years	15 years	11 years	11 years	9 years	9 years	10 years	16 years
Sex	Female	Female	Female	Male	Male	Male	Female	Male	Female
Race/ethnicity	White/ non-Hispanic	White/Hispanic	Asian/non-Hispanic	African American/ non-Hispanic	White/ non-Hispanic	White/ non-Hispanic	Other/Hispanic	Other/Hispanic	White/ non-Hispanic
Kidney biopsy diagnosis	DDD (with crescents)	C3GN	C3GN	C3GN	DDD	Indeterminate	DDD (with crescents)	C3GN (with TMA)	DDD
Presenting signs/symptoms	Seizure, gross hematuria	Nephrotic syndrome	Proteinuria	Seizure (PRES), hypertension, nephrotic syndrome	Gross hematuria	Nephrotic syndrome	Hypertension, nephrotic syndrome	Gross hematuria, nephrotic syndrome	Nephrotic syndrome
eGFRa at onset	30	97	126	203	106	93	63	125	93
Hematuria	Present	Present	Present	Present	Present	Present	Present	Present	Present
Proteinuria (Urine protein to creatinine ratio: g/g)	Present (UPC 4.3)	Present (UPC 6.7)	Present (UPC 1.7)	Present (UPC 5.6)	Present (UPC 1.8)	Present (UPC 0.5)	Present (UPC 7.5)	Present (UPC 3.0)	Present (UPC 7.3)
Albumin (Ref, > 3.5 g/dL)	3.5	2.8	3.5	1.6	3.4	2.9	1.4	2.3	1.9
C3b (Ref, > 70 mg/dL)	18 (L)	46 (L)	48 (L)	7 (L)	< 4 (L)	13 (L)	49 (L)	< 12 (L)	104 (N)
C4b (Ref, > 11 mg/dL)	20 (N)	15 (N)	27 (N)	15 (N)	22 (N)	18 (N)	31 (N)	21 (N)	25 (N)
Other serologies	ANCA (−)	ND	ANA + (1:640) ANCA (−)	ANA (−)	ANA (−)	ANA + (1:160)	ANA (−) ANCA (−)	ND	ANA (−)
Immunosuppression	Steroids (IV methylpred 10 mg/kg × 5 doses; prednisone 2 mg/kg daily followed by taper) MMF (600 mg/m^2 BID × 1 month then 300 mg/m^2 BID)	None	Steroids (prednisone 0.5 mg/kg every other day) MMF (450 mg/m^2 BID)	MMF (400 mg/m^2)	Steroids (IV methylpred 10 mg/kg × 3 doses; prednisone 1 mg/kg every other day) MMF (330 mg/m^2 BID; increased to 600 mg/m^2 BID)	None	MMF (500 mg/m^2 BID)	Steroids (prednisone 1 mg/kg every other day) MMF (200 mg/m^2 BID) Tacrolimus (2–4 mg BID)	Steroids (prednisone 1 mg/kg every other day)
Time to final follow up (months)	6	15	27	28	21	36	120	46	2
eGFR* at final follow up	130	103	104	170	136	108	20	49	72
Urinalysis at final follow up	Persistent proteinuria and hematuria	Resolved proteinuria and hematuria	Persistent proteinuria (improved); resolved hematuria	Persistent proteinuria (unchanged) and hematuria	Persistent proteinuria (worse) and hematuria	Persistent proteinuria (unchanged) and hematuria	Persistent proteinuria and hematuria (prior to transplant)	Persistent proteinuria (unchanged) and hematuria	Persistent proteinuria (improved) and hematuria
Renal replacement therapy	No	No	No	No	No	No	Yes	No	No

[a] eGFR calculated by revised Schwartz equation; ml/min/1.73 m^2

[b] Serum C3 and C4 performed by institutional lab with internal reference standards

ANA anti-nuclear antibodies, *ANCA* anti-neutrophil cytoplasmic autoantibody, *C3GN* C3 glomerulonephritis, *DDD* dense deposit disease, *L* low, *N* normal, *ND* not done, *MMF* mycophenolate mofetil, *PRES* posterior reversible encephalopathy syndrome, *TMA* thrombotic microangiopathy

Table 2 Biopsy findings in patients with C3 glomerulopathy

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Diagnosis	DDD	C3GN	C3GN	C3GN	DDD	Indeterminate	DDD	C3GN, TMA	DDD
Light microscopy pattern of Injury	DEPGN, focal (10%) necrotizing crescents	MPGN	MPGN	MPGN	MPGN	MPGN	MPGN, focal (30%) crescents	MPGN	MPGN
Number of GS glomeruli/total glomeruli	0/81	1/52	1/28	2/68	0/86	0/61	0/24	2/12	7/27
IFTA %	0	10	0	0	0	0	10%	0	10%
Immunofluorescence	C3 (4+), IgM (1+)	C3 (2–3+), Ig negative	C3 (3+), Ig negative	C3 (4+), IgG (2+), C1q (2+),	C3 (4+), IgM (1+)	C3 (4+), IgG (1+), IgM (1+)	C3 (4+), IgM (2+)	C3 (3+), IgG (1+), IgM (1+), C1q (1+)	C3 (2+), Ig negative
Electron Microscopy	Subepithelial, dense intra-membranous, and mesangial deposits	Subendothelial, intra-- membranous, and mesangial deposits	Subepithelial, intra-membranous, subendothelial, and mesangial deposits	Subepithelial, intra-membranous, subendothelial, and mesangial deposits	Subendothelial, dense intra-membranous, and mesangial deposits	Subepithelial, dense intra-membranous, subendothelial, and mesangial deposits	Subepithelial, dense intra-membranous, and subendothelial deposits	Subepithelial, subendothelial, and mesangial deposits; fibrin tactoids and subendothelial flocculent material	Dense intra-membranous and mesangial deposits

C3GN C3 glomerulonephritis, *DDD* dense deposit disease, *DEPGN* diffuse endocapillary proliferative glomerulonephritis, *GS* globally sclerosed, *IFTA* interstitial fibrosis and tubular atrophy, *MPGN* membranoproliferative glomerulonephritis, *TMA* thrombotic microangiopathy

Results

Clinical features and laboratory findings

Out of 589 total native kidney biopsies examined from April 2007 through February 2017, eleven biopsy specimens from nine patients met inclusion criteria. Four patients (1 male, 3 female) had DDD, four patients (2 male, 2 female) had C3GN, and one patient (male) was deemed indeterminate based upon overlapping features seen by EM. The clinical and laboratory findings are described in Table 1. Of the 9 patients included in our study, mean age at diagnosis was 11 years (range 8–16), and 55% were females. All patients presented with hematuria and proteinuria. At onset, 2 patients with DDD presented with decreased kidney function (eGFR < 90 ml/min/1.73 m^2). The mean follow-up was 33 months (range 2–120). Three patients showed progressive renal dysfunction (defined as declining eGFR < 90 ml/min/1.73 m^2), including 2 patients with DDD (1 of whom required a kidney transplant) and 1 patient with C3GN and thrombotic microangiopathy (TMA) on the patient's initial biopsy. Upon review of available records for this patient (including CBC done at the time of the biopsy and serial CBCs over the next 24 months), the patient had mild anemia (Hgb ranging from 10 to 12) without thrombocytopenia (platelets ranged from 233,000 to 320,000), thus lacking clinical manifestations of TMA.

Kidney biopsy findings

The complete kidney biopsy findings are summarized in Table 2. Six cases had weakly positive Ig staining by IF, but all met inclusion criteria with dominant C3 IF staining. EM studies showed mesangial and glomerular capillary wall electron-dense deposits in all biopsies of C3GN, and highly electron-dense intra-membranous deposits in all biopsies of DDD. Chronicity was minimal with all cases showing 0–10% interstitial fibrosis and tubular atrophy. All initial biopsies at our institution showed a membranoproliferative pattern of injury, except patient 1 whose biopsy showed a diffuse endocapillary proliferative and exudative glomerulonephritis with focal (10%) necrotizing crescents and medullary angiitis by light microscopy; representative kidney biopsy findings are shown in Figs. 1 and 2. Medullary angiitis has been reported in other causes of acute glomerulonephritis, but not in C3G to our knowledge [19].

Patient 7 also had focal crescents. C3G with a crescentic phenotype has been previously described [10]; however, the extent of crescent formation they reported was greater than that seen in our two patients.

Patient 8 had features of TMA by EM, including fibrin tactoids and expansion of subendothelial spaces by electron-lucent material (Fig. 2d). Features of TMA were not seen by light microscopy.

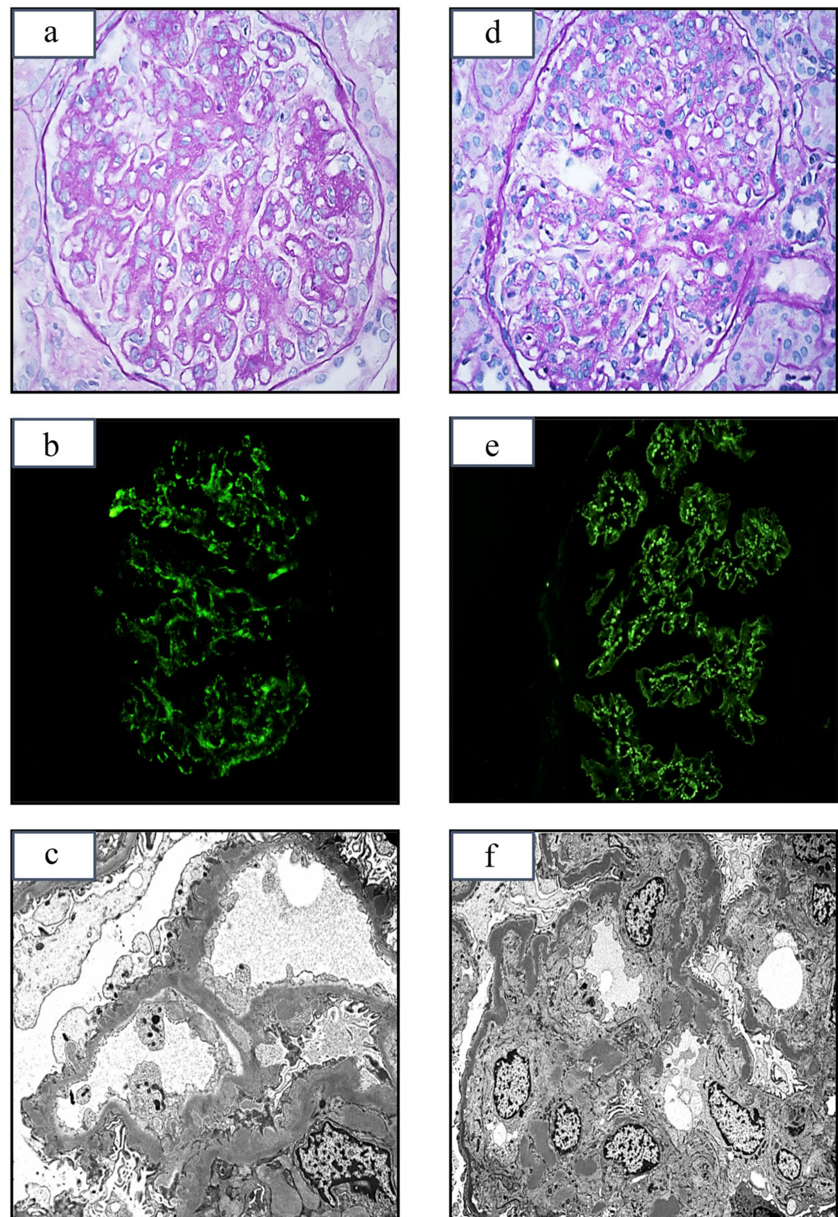

Fig. 1 Kidney biopsy findings in C3GN (**a**–**c**, patient 4) and DDD (**d**–**f**, patient 5). **a** Light microscopy showing membranoproliferative pattern of injury (PAS, original magnification × 400), **b** immunofluorescence microscopy shows granular staining in the mesangium and segmentally along the capillary walls for C3 (IF, × 400), **c** electron micrograph shows numerous subepithelial electron-dense deposits, many of which appear to replace areas of basement membranes without clearly defined borders. A few subendothelial and mesangial electron-dense deposits are also seen (EM, × 1800) **d** light microscopy showing membranoproliferative pattern of injury (PAS, original magnification × 400), **e** immunofluorescence microscopy shows band-like staining of capillary walls and granular staining in mesangium with ring-shaped forms in some areas for C3 (IF, × 400), **f** electron micrograph shows linear band-like electron-dense transformation of the lamina densa and rounded electron densities within the mesangium (EM, × 1200)

Patient 6 was classified as "indeterminate" due to EM, which showed subepithelial, subendothelial, and mesangial electron-dense deposition in addition to segmental ribbon-like highly electron-dense intra-membranous deposits.

Infection-related glomerulonephritis was clinically suspected in three patients prior to biopsy based upon clinical symptoms or laboratory parameters; however in these patients, the C3 remained persistently low at least 6 weeks or greater and was the indication for biopsy. The presence of subepithelial "hump-shaped" deposits was identified by electron microscopy in three patients (cases 1, 4, and 6). Streptococcal infection may precede the development of C3G and "hump-shaped" subepithelial deposits are commonly seen in both infection-related GN and C3G [1, 20].

Patients 7 and 8 received follow-up biopsies at 72 and 29 months post initial biopsy, respectively. Repeat biopsies showed proliferative features and only patient 7's biopsy showed significant disease progression, with chronic injury characterized by severe interstitial fibrosis and tubular atrophy. Patient 7's allograft biopsy 30 days post-transplant showed no evidence of recurrent disease.

Evaluation of complement profiles and genetic testing

Four patients had extensive complement-profiling performed, which revealed variable abnormalities in the AP as shown in Table 3. Five patients in this study had limited or no

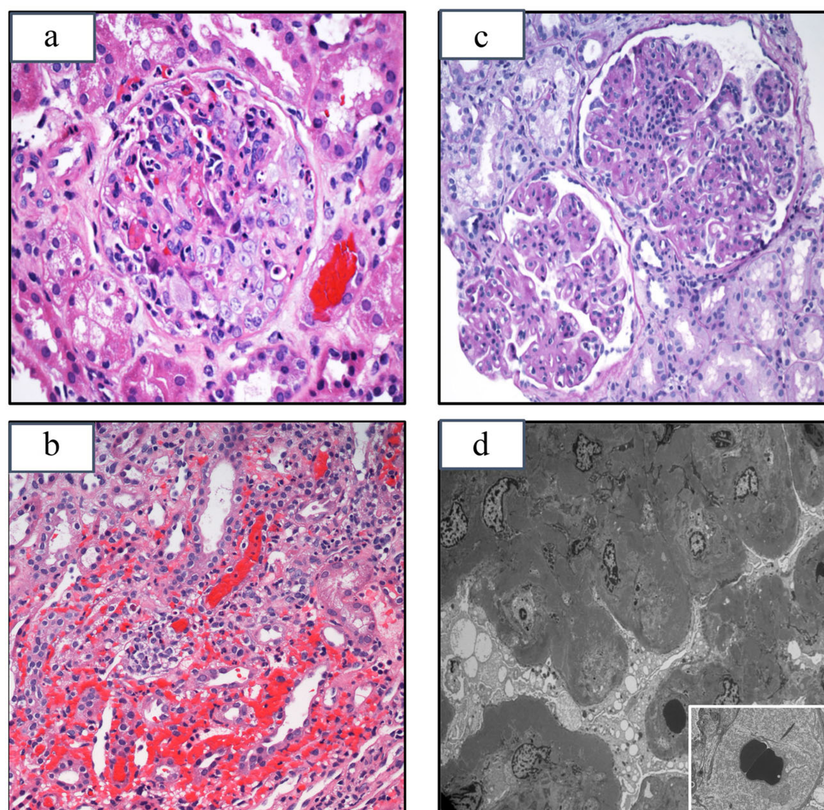

Fig. 2 Kidney biopsy findings in DDD with medullary angiitis (**a**, **b**; patient 1), and C3GN with TMA (**c**, **d**; patient 8). **a** Light microscopy showing membranoproliferative pattern of injury with a cellular crescent (H&E, original magnification × 400), **b** light microscopy showing extensive interstitial hemorrhage with associated interstitial neutrophils and karyorrhectic debris (H&E, original magnification × 200), **c** light microscopy showing membranoproliferative pattern of injury (PAS, original magnification × 200), **d** electron micrograph showing numerous ill-defined, confluent subepithelial, subendothelial, and mesangial deposits; inset shows subendothelial electron-lucent, flocculent material admixed with a fibrin tactoid (EM, × 700; inset × 3500)

complement evaluation including patient 7 who was the only one to have CFH autoantibodies.

Genetic testing done in 5 of the 9 patients failed to identify any causative mutations. Of note, 3 patients had variants of unknown significance (VUS) identified. Specifically, patient 2 had a heterozygous VUS in the gene *ADAMTS13* (c.3287G>A,p.Arg1096His) not predicted to be pathogenic, and patient 4 also had a heterozygous VUS in gene *ADAMTS13* (c.2936G>A,p.Arg979Gln) predicted to be pathogenic. Patient 8 had a heterozygous variant in the gene *CFI* (c.1354G>A,p.Ala452Thr) reported as a VUS though predicted to be pathogenic. Mutations in *CFI*, along with *CFH* and membrane cofactor protein (*MCP*) genes, have been described in association with C3G and atypical hemolytic-uremic syndrome (aHUS) [7, 21, 22].

Treatment and follow-up

All patients were treated with renin-angiotensin blockade (either with ACE inhibitors or angiotensin receptor II blockers), with 2 patients (patients 2 and 6) treated solely with these agents without disease progression. Six patients (patients 1, 3, 4, 5, 8, and 9) received steroids, four of whom (patients 1, 3, 5, and 8) were additionally treated with mycophenolate mofetil (MMF) at their initial diagnosis. Patient 8 also received tacrolimus, with doses outlined in Table 1.

Follow-up ranged from 2 months to 10 years after initial renal biopsy with a mean follow-up of 11 months. Patient 1 presented with significant renal impairment (eGFR of 30 ml/min/1.73 m^2) with DDD and crescents on her initial biopsy; however, after treatment with steroids and MMF, her kidney function normalized. Three patients, two with DDD (patients 7 and 9) and one with C3GN (patient 8) showed significant decline in renal function. Despite treatment with steroids, MMF, and rituximab, patient 7 developed end-stage kidney disease, requiring peritoneal dialysis for 32 months until receiving a deceased donor renal allograft. Patient 9, also with DDD, was treated with steroids alone and showed progressive kidney disease, with eGFR declining from 93 ml/min/1.73 m^2 to 72 ml/min/1.73 m^2 over a period of 2 months. Patient 8, with C3GN, was treated with steroids, MMF, prograf, and rituximab, with continued proteinuria and decline in renal function at last follow-up from 125 ml/min/1.73 m^2 to 49 ml/min/1.73 m^2 (46 months).

Discussion

In this study, we describe the clinical features, kidney biopsy findings, complement abnormalities, and course of 9 pediatric patients with C3G. Since the entity of C3G was defined in 2013, a complete evaluation with genetic testing, autoantibody screening, and complement function assays are routinely

Table 3 Complement profile and genetic testing in patients with C3 glomerulopathy

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Diagnosis	DDD (with crescents)	C3GN	C3GN	C3GN	DDD	Indeterminate	DDD (with crescents)	C3GN (with TMA)	DDD
CH50	33 U/ml Ref 30–90[a]	ND	48 U/ml Ref 30–90[a]	15 U/ml Ref 30–90[a]	12 U/ml Ref 30–90[a]	34 units Ref 60–144[b]	ND	ND	ND
Alternative Pathway Functional Assay	69% Ref 50–130%[c]	ND	49% Ref 50–130%[c]	0% Ref 50–130%[c]	2% Ref 50–130%[c]	3 U/mL Ref 77–159[d]	ND	ND	ND
Hemolytic Assay	12.4% Ref < 3%[e]	ND	1.7% Ref < 3%[e]	0.2% Ref < 3%[e]	0.9% Ref < 3%[e]	ND	ND	ND	ND
FB Autoantibody	78 AU Ref < 200[f]	ND	253 AU Ref < 200[f]	192 AU Ref < 200[f]	197 AU Ref < 200[f]	ND	ND	ND	ND
FH Autoantibody	< 50 AU Ref < 200[g]	ND	< 50 AU Ref < 200[g]	< 50 AU Ref < 200[g]	< 50 AU Ref < 200 [g]	ND	1:100 Ref < 1:50[h]	ND	ND
C3 NeF Assay – IFE	27.5% Ref < 7.5%[i]	ND	0.35 Ref < 0.3[j]	4.2% Ref < 7.5%[i]	51.8% Ref < 7.5%[i]	ND	High Ref < 0.3[j]	0.19 Ref < 0.3[j]	ND
C3 NeF – C3CSA	100% Ref < 20%[k]	ND	ND	41% Ref < 20%[k]	119% Ref < 20%[k]	ND	ND	ND	ND
C5 NeF – C3CSAP	79% Ref < 20%[l]	ND	10% Ref < 20%[l]	49% Ref < 20%[l]	90% Ref < 20%[l]	ND	ND	ND	ND
C4 NeF	9% Ref < 20%[m]	ND	132% Ref < 20%[m]	57% Ref < 20%[m]	12% Ref < 20%[m]	ND	ND	ND	ND
C3 level	0.5 g/L Ref 0.9–18[n]	ND	1.0 g/L Ref 0.9–18[n]	0.16 g/L Ref 0.9–18[n]	0.16 g/L Ref 0.9–18[n]	ND	ND	ND	ND
C4 level	0.22 g/L Ref 0.15–0.57[o]	ND	ND	ND	ND	ND	ND	ND	ND
C3d	ND	ND	0.73 mg/L Ref < 0.7[p]	0.97 mg/L Ref < 0.7[p]	1.03 mg/L Ref < 0.7[p]	ND	ND	ND	ND
FB level	20.3 mg/dL Ref 22–50[q]	ND	313 mcg/mL Ref 127–278[r]	36 mg/dL Ref 22–50[q]	41.9 mg/dL Ref 22–50[q]	191 mcg/mL Ref 127–278[q]	185 mcg/mL Ref 127–278[q]	ND	ND
Ba Fragment Level	1.8 mg/L Ref < 1.2[s]	ND	0.6 mg/L Ref < 1.2[s]	0.4 mg/L Ref < 1.2[s]	0.8 mg/L Ref < 1.2[s]	ND	ND	ND	ND
Bb Fragment Level	1.3 mg/L Ref < 2.2[t]	ND	ND	1.8 mg/L Ref < 2.2[t]	2.8 mg/L Ref < 2.2	ND	ND	ND	ND
Properdin	13.1 mg/L Ref 10–33[u]	ND	14.6 mg/L Ref 10–33[u]	8.4 mg/L Ref 10–33[u]	13.1 mg/L Ref 10–33[u]	ND	ND	ND	ND
C5 level	8.8 mg/L Ref 10–21[v]	ND	13.5 mg/L Ref 10–21[v]	3.5 mg/L Ref 10–21[v]	10.2 mg/L Ref 10–21[v]	ND	ND	ND	ND
Soluble C5b-9	0.23 mg/L Ref < 0.3[w]	ND	358 ng/mL Ref 72–244[x]	3.8 mg/L Ref < 0.3[w]	0.77 mg/L Ref < 0.3[w]	ND	ND	ND	ND
FH level	192 mg/L Ref 180–420[y]	ND	297 mcg/mL Ref 160–412[z]	ND	ND	ND	232 mcg/mL Ref 160–412[z]	ND	ND
FI level	33.7 mg/L Ref 16–40[aa]	ND	46.8 mcg/mL Ref 29.3–58.5[bb]	ND	ND	ND	ND	ND	ND
Genetic Evaluation	ND	No causative variants (negative for CFH, CFI, MCP, CFB, CFHR5, C3, THBD, DGKE, PLG, MMACHC) heterozygous VUS in ADAMTS13	ND	No causative variants (negative for CFH; CFI, MCP/CD46, CFB, CFHR5, C3, THBD, DGKE, PLG; heterozygous VUS in ADAMTS13	No causative variants (negative for CFH, CFI, MCP, CFB, CFHR5, C3, THBD, DGKE, PLG, ADAMTS13)	ND	No causative variants (negative for CFH, CFI, MCP, CFB, CFHR5, C3, THBD, DGKE, PLG, ADAMTS13)	Limited evaluation (MCP, CFB, CFH normal); heterozygous VUS in CFI)	ND

[a] CH50 testing: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: 30–90 U/ml

[b] CH50 testing: ARUP Laboratories, Salt Lake City, UT, Reported reference range: 60–144 units

[c] Alternative Pathway Functional Assay: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: 50–130%

[d] Alternative Pathway Functional Assay: National Jewish Laboratories, Denver, CO; Reported reference range: 77–159 U/mL

[e] Hemolytic Assay: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 3%

[f] FB Autoantibody: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 200 AU

[g] FH Autoantibody: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 200 AU

[h] FH Autoantibody: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: titer < 1:50

[i] C3 NeF Assay – IFE: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 7.5%

[j] C3 NeF Assay – C3CSA: National Jewish Laboratories, Denver, CO; Reported reference range: < 0.3

[k] C3 NeF Assay – C3CSA: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 20%

[l] C5 NeF Assay – C3CSAP: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 20%

[m] C4 NeF Assay: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 20%

[n] Plasma C3 level: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: 0.9–18 g/L

[o] Plasma C4 level: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: 0.15–0.57 g/L

[p] C3d level: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 0.7 mg/L

[q] FB level: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: 22–50 mg/dL

[r] FB level: National Jewish Laboratories, Denver, CO; Reported reference range: 127–278 mcg/mL

[s] Ba level: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 1.2 mg/L

[t] Bb level: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 2.2 mg/L

[u] Properdin level: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: 10–33 mg/L

[v] C5 level: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: 10–21 mg/L

[w] Sc5b-9 level: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: < 0.3 mg/L

[x] Sc5b-9 level: National Jewish Laboratories, Denver, CO; Reported reference range: 72–244 ng/mL

[y] Factor H: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: 180–420 mg/L

[z] Factor H level: National Jewish Laboratories, Denver, CO; Reported reference range: 160–412 mcg/mL

[aa] Factor I: Molecular Otolaryngology & Renal Research Laboratories, Clinical Diagnostic Services, Iowa City, IA; Reported reference range: 16–40 mg/L

[bb] Factor I level: National Jewish Laboratories, Denver, CO; Reported reference range: 29.3–58.5 mcg/mL

C3GN C3 glomerulonephritis, *CFB* cofactor B, *CFH* cofactor H, *CFHR5* cofactor H-related 5, *CFI* cofactor I, *CH50* complement hemolytic 50, *CSA* convertase surface assay, *CSAP* convertase surface assay with properdin, *DDD* dense deposit disease, *DGKE* diacylglycerol kinase epsilon, *FB* factor B, *FH* factor H, *IFE* immunofixation electrophoresis, *MCP* membrane cofactor protein, *MMACHC* metabolism of cobalamin associated C, *ND* not done, *NeF* nephritic factor, *PLG* plasminogen, THBD thrombomodulin, *TMA* thrombotic microangiopathy, *VUS* variant of unknown significance. See additional information in supplementary material.

recommended. However, how these findings correlate with prognosis, response to treatment, and variations observed on renal biopsy remain unknown, particularly in the pediatric population.

C3G is defined by histopathological findings of dominant glomerular C3 deposition (two orders of magnitude greater than any other immune reactant); other glomerular diseases may occasionally satisfy “C3-dominant deposition with scanty immunoglobulins” including post-infectious glomerulonephritis (PIGN) [23]. Our study re-examined 589 biopsies and found one patient that was re-classified as C3G following our review. Also, several biopsies in this small case series identified unusual pathological findings after detailed review of the initial biopsies, highlighting the diagnostic challenges of this disease.

Previous studies have identified causative genetic variants and/or acquired factors that dysregulate the complement AP in only a subset of patients, leaving the etiology of disease unknown for many [24]. We report evidence of complement dysregulation in all 6 patients that underwent complement profile testing. Of the 5 patients who underwent genetic testing, no causative variants were identified. Now that this testing is more readily available, clinicians must be versed in ordering and interpreting such studies. While complement profile abnormalities were detected, no single marker was consistent across all patients tested. Larger studies with extensive complement testing are necessary to investigate the clinical significance of individual markers, and further studies are needed to understand if the presence of multiple autoantibodies/nephritic factors are clinically and/or prognostically significant. As previously reportedly, one of the most significant pathogenic factors in C3G is autoantibody against the C3 convertase of the AP (C3NeF): subsequent studies have shown additional nephritic factors driving disease. Although our sample size is small and not all patients were screened for C4NeF, two patients with C3GN were found to have the presence of both C3Nef and C4NeF. Zhang et al. studied 168 adults (age range 17–44 years) with C3G and found C4NeF present in approximately 3%, including 3 with C3GN and 1 with DDD. In the C4NeF positive patients with C3GN, 1 had autoantibodies to FH, and 1 was positive for C3NeF [3]. A recent study by Marinozzi et al. investigated C3NeF and C5NeF in C3G. The authors found that out of 59 tested, 29% were positive for C3NeF only, 10% were positive for C5NeF only, and 39% were positive for both. The authors found that children who were double positive had a better outcome than those who were double negative [25]. Two of our DDD patients (1 and 5) were also double positive. Both had persistent hematuria and proteinuria but saw improvement in eGFR.

There are several limitations to this study. The sample size is small compared to studies that include pediatric and adult patients. Currently, there is no standardized treatment for C3G and with highly variable outcomes; one cannot make predictions regarding prognostic factors for kidney disease progression. Given that it was done retrospectively, genetics and complement profiles were not completed on all patients reported. While established clinical laboratories were utilized for complement studies and genetic testing, this was not standardized, and some tests were not performed in all of the patients.

In conclusion, we present an entirely pediatric cohort of C3G patients, including their clinical presentation, renal biopsy features, results of complement investigation studies, and renal outcome. Genetic testing, autoantibody assays, and complement pathway functional assays are now routinely recommended in the evaluation of patients with a histopathological diagnosis of C3G. The outcome of patients with C3G at this time remains heterogeneous with poor predictive markers for progression of kidney disease. Thus, large, prospective, multi-institutional studies are needed to elucidate the prognostic and therapeutic implications of these varied complement abnormalities of this rare disease in children.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study as applicable.

References

1. Pickering MC, D'Agati VD, Nester CM, Smith RJ, Haas M, Appel GB, Alpers CE, Bajema IM, Bedrosian C, Braun M, Doyle M, Fakhouri F, Fervenza FC, Fogo AB, Fremeaux-Bacchi V, Gale DP, Goicoechea de Jorge E, Griffin G, Harris CL, Holers VM, Johnson S, Lavin PJ, Medjeral-Thomas N, Paul Morgan B, Nast CC, Noel LH, Peters DK, Rodriguez de Cordoba S, Servais A, Sethi S, Song WC, Tamburini P, Thurman JM, Zavros M, Cook HT (2013) C3 glomerulopathy: consensus report. Kidney Int 84: 1079–1089
2. Hou J, Markowitz GS, Bomback AS, Appel GB, Herlitz LC, Barry Stokes M, D'Agati VD (2014) Toward a working definition of C3 glomerulopathy by immunofluorescence. Kidney Int 85:450–456
3. Zhang Y, Meyer NC, Fervenza FC, Lau W, Keenan A, Cara-Fuentes G, Shao D, Akber A, Fremeaux-Bacchi V, Sethi S, Nester CM, Smith RJH (2017) C4 nephritic factors in C3 glomerulopathy: a case series. Am J Kidney Dis 70:834–843
4. Medjeral-Thomas NR, O'Shaughnessy MM, O'Regan JA, Traynor C, Flanagan M, Wong L, Teoh CW, Awan A, Waldron M, Cairns T, O'Kelly P, Dorman AM, Pickering MC, Conlon PJ, Cook HT (2014) C3 glomerulopathy: clinicopathologic features and predictors of outcome. Clin J Am Soc Nephrol 9:46–53
5. Sethi S, Fervenza FC, Zhang Y, Zand L, Vrana JA, Nasr SH, Theis JD, Dogan A, Smith RJ (2012) C3 glomerulonephritis:

clinicopathological findings, complement abnormalities, glomerular proteomic profile, treatment, and follow-up. Kidney Int 82:465–473
6. Tesar V, Hruskova Z (2017) Immunosuppressive treatment in C3 glomerulopathy: time to reconsider our approach. Am J Nephrol 46:93–95
7. Barbour TD, Pickering MC, Cook HT (2013) Recent insights into C3 glomerulopathy. Nephrol Dial Transplant 28:1685–1693
8. Haffner K, Michelfelder S, Pohl M (2015) Successful therapy of C3Nef-positive C3 glomerulopathy with plasma therapy and immunosuppression. Pediatr Nephrol 30:1951–1959
9. Riedl M, Thorner P, Licht C (2017) C3 glomerulopathy. Pediatr Nephrol 32:43–57
10. Ravindran A, Fervenza FC, Smith RJH, Sethi S (2017) C3 glomerulonephritis with a severe crescentic phenotype. Pediatr Nephrol 32:1625–1633
11. Kawasaki Y, Kanno S, Ono A, Suzuki Y, Ohara S, Sato M, Suyama K, Hashimoto K, Hosoya M (2016) Differences in clinical findings, pathology, and outcomes between C3 glomerulonephritis and membranoproliferative glomerulonephritis. Pediatr Nephrol 31:1091–1099
12. Okuda Y, Ishikura K, Hamada R, Harada R, Sakai T, Hamasaki Y, Hataya H, Fukuzawa R, Ogata K, Honda M (2015) Membranoproliferative glomerulonephritis and C3 glomerulonephritis: frequency, clinical features, and outcome in children. Nephrology (Carlton) 20:286–292
13. Vernon KA, Goicoechea de Jorge E, Hall AE, Fremeaux-Bacchi V, Aitman TJ, Cook HT, Hangartner R, Koziell A, Pickering MC (2012) Acute presentation and persistent glomerulonephritis following streptococcal infection in a patient with heterozygous complement factor H-related protein 5 deficiency. Am J Kidney Dis 60:121–125
14. Lebreton C, Bacchetta J, Dijoud F, Bessenay L, Fremeaux-Bacchi V, Sellier-Leclerc AL (2017) C3 glomerulopathy and eculizumab: a report on four paediatric cases. Pediatr Nephrol 32:1023–1028
15. Xiao X, Ghossein C, Tortajada A, Zhang Y, Meyer N, Jones M, Borsa NG, Nester CM, Thomas CP, de Cordoba SR, Smith RJ (2016) Familial C3 glomerulonephritis caused by a novel CFHR5-CFHR2 fusion gene. Mol Immunol 77:89–96
16. Servais A, Noel LH, Roumenina LT, Le Quintrec M, Ngo S, Dragon-Durey MA, Macher MA, Zuber J, Karras A, Provot F, Moulin B, Grunfeld JP, Niaudet P, Lesavre P, Fremeaux-Bacchi V (2012) Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. Kidney Int 82:454–464
17. Bomback AS, Santoriello D, Avasare RS, Regunathan-Shenk R, Canetta PA, Ahn W, Radhakrishnan J, Marasa M, Rosenstiel PE, Herlitz LC, Markowitz GS, D'Agati VD, Appel GB (2018) C3 glomerulonephritis and dense deposit disease share a similar disease course in a large United States cohort of patients with C3 glomerulopathy. Kidney Int 93:977–985
18. Walker PD, Cavallo T, Bonsib SM, Ad Hoc Committee on Renal Biopsy Guidelines of the Renal Pathology S (2004) Practice guidelines for the renal biopsy. Mod Pathol 17:1555–1563
19. Hendricks AR, Harris AA, Walker PD, Larsen CP (2013) Renal medullary angiitis: a case series from a single institution. Hum Pathol 44:521–525
20. Nasr SH, Valeri AM, Appel GB, Sherwinter J, Stokes MB, Said SM, Markowitz GS, D'Agati VD (2009) Dense deposit disease: clinicopathologic study of 32 pediatric and adult patients. Clin J Am Soc Nephrol 4:22–32
21. Servais A, Fremeaux-Bacchi V, Lequintrec M, Salomon R, Blouin J, Knebelmann B, Grunfeld JP, Lesavre P, Noel LH, Fakhouri F (2007) Primary glomerulonephritis with isolated C3 deposits: a new entity which shares common genetic risk factors with haemolytic uraemic syndrome. J Med Genet 44:193–199
22. Kavanagh D, Kemp EJ, Mayland E, Winney RJ, Duffield JS, Warwick G, Richards A, Ward R, Goodship JA, Goodship TH (2005) Mutations in complement factor I predispose to development of atypical hemolytic uremic syndrome. J Am Soc Nephrol 16:2150–2155
23. Sethi S, Fervenza FC, Zhang Y, Zand L, Meyer NC, Borsa N, Nasr SH, Smith RJ (2013) Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement. Kidney Int 83:293–299
24. Ito N, Ohashi R, Nagata M (2017) C3 glomerulopathy and current dilemmas. Clin Exp Nephrol 21:541–551
25. Marinozzi MC, Chauvet S, Le Quintrec M, Mignotet M, Petitprez F, Legendre C, Cailliez M, Deschenes G, Fischbach M, Karras A, Nobili F, Pietrement C, Dragon-Durey MA, Fakhouri F, Roumenina LT, Fremeaux-Bacchi V (2017) C5 nephritic factors drive the biological phenotype of C3 glomerulopathies. Kidney Int 92:1232–1241

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.