C3 Glomerulopathies

25

Christoph Licht, Marina Vivarelli,
and Sanjeev Sethi

Pathology of C3 Glomerulopathies

Membranoproliferative Glomerulonephritis

Membranoproliferative glomerulonephritis (MPGN) refers to a pattern of injury occurring as response to deposition of immunoglobulins (Ig)/immune-complexes (IC) and/or complement factors in the mesangium and/or along the glomerular capillary walls [1]. The deposition of Ig/IC and complement factors results in an inflammatory response due to: (1) proliferation of indigenous glomerular cells such as mesangial cells, endothelial cells, and infiltration and proliferation of leukocytes, and (2) synthesis of matrix material such as mesangial matrix material, basement membrane material and fibrin. The MPGN pattern is thus characterized by (1) increased cellularity in the mesangium (mesangial proliferation) and within the capillary lumen (endocapillary proliferation), and (2) mesangial expansion by matrix material and capillary wall remodelling with formation of double contours. The MPGN pattern is distinguished from a diffuse (endocapillary) proliferative pattern by the finding that in MPGN at the time of diagnosis the injury is often chronic, resulting in a healing or remodeling phase in the mesangium (mesangial expansion, often with nodule formation) and along the glomerular capillaries (double contour formation) [2].

Glomerular deposition of Ig/IC originates from three basic pathogenic mechanisms (see later in this chapter Fig. 25.3a–d): (1) deposition of monoclonal Ig as a result of a monoclonal gammopathy due to a plasma cell or B cell disorder [3–6]; (2) deposition of antigen-antibody or IC as a result of an infection [7]; and (3) IC deposition as a result of an autoimmune disease [8, 9]. Immunofluorescence studies can often confirm the underlying pathogenic mechanism of Ig/IC deposition based on the type of Ig detected. Complement factors are also noted along with the Ig/IC, due to activation of the complement system via the *classical pathway* by the Ig/IC. On the other hand, glomerular deposition of complement factors alone or in the presence of scant Ig results from dysregulation of the *complement alternative pathway* (*CAP*). The term C3 glomerulopathy (C3G) is used to define this entity [10].

C. Licht (✉)
Department of Nephrology,
The Hospital for Sick Children,
555 University Avenue, Toronto M5G 1X8, Canada
e-mail: christoph.licht@sickkids.ca

M. Vivarelli
Department of Nephrology and Dialysis,
Ospedale Pediatrico Bambino Gesu,
Pizza S.Onofrio 4, Rome 00165, Italy
e-mail: marina.vivarelli@opbg.net

S. Sethi
Laboratory Medicine and Pathology Department,
Mayo Clinic, 200 1st Street SW,
Rochester, MN 55902, USA
e-mail: sethi.sanjeev@mayo.edu

D.F. Geary, F. Schaefer (eds.), *Pediatric Kidney Disease*, DOI 10.1007/978-3-662-52972-0_25

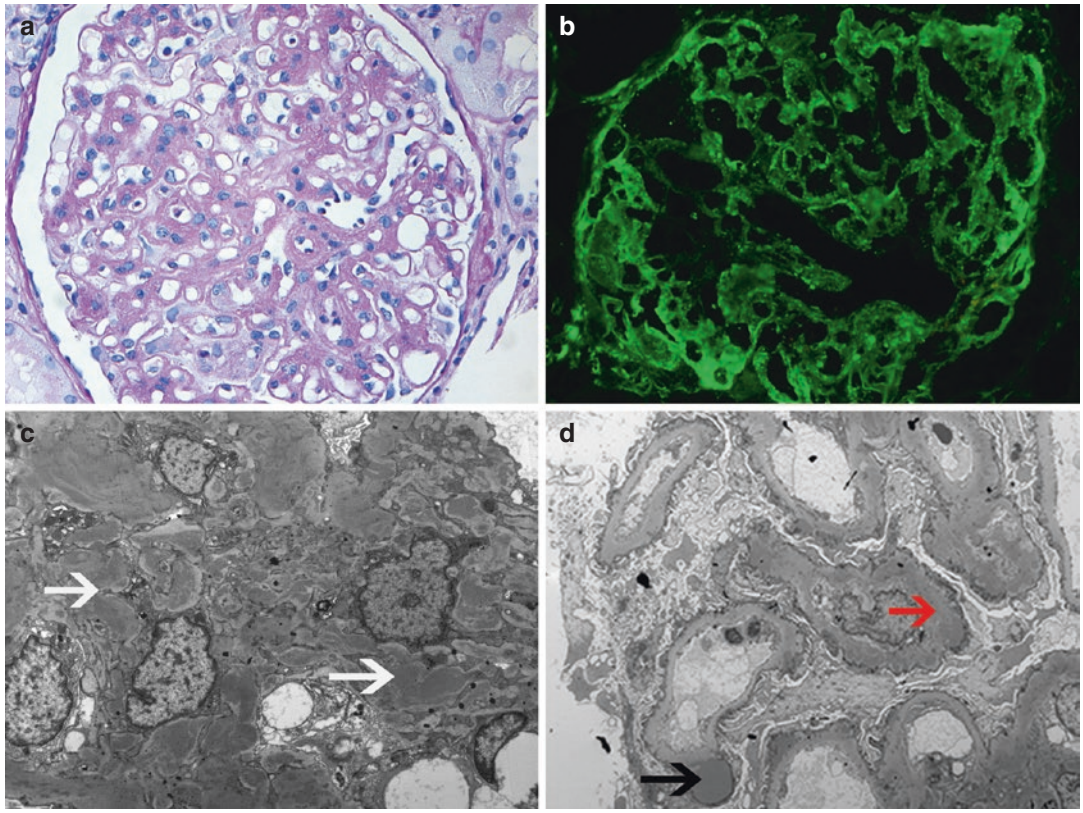

Fig. 25.1 C3 glomerulonephritis. (**a**) Light microscopy showing a MPGN pattern of injury (periodic acid Schiff 60×). (**b**) Immunofluorescence microscopy showing mesangial and capillary wall staining for C3. Immunofluorescence studies were negative for Igs. (**c**, **d**) EM showing (**c**) Numerous mesangial electron dense deposits (*white arrows*) and (**d**) Capillary wall deposits (*red arrow*-intramembranous and subendothelial deposits, *black arrow*-subepithelial deposit/hump) (**c** 4,200×, **d** 2,500)

Based on these findings MPGN has recently been classified into- Ig/IC-mediated glomerulonephritis and complement-mediated glomerulonephritis (C3 glomerulopathy, C3G) [11]. Thus, immunofluorescence studies of the kidney biopsy are the key to the classification of MPGN into Ig/IC-mediated or complement-mediated MPGN.

C3 Glomerulopathy

While the pathogenesis of MPGN includes a variety of causes, the pathogenesis of C3G is now thought to be predominantly linked to defects in the control of the complement system, in particular its alternative pathway (CAP). Deposition of complement factors in the mesangium and/or along the glomerular capillary walls results in a proliferative glomerulonephritis. The term "C3 glomerulopathy" (C3G) is now used to define the entity of a glomerulonephritis characterized by C3 accumulation, with absent or scanty Ig deposition [10, 12, 13]. C3G encompasses the entities of C3 glomerulonephritis (C3GN) and dense deposit disease (DDD) [10, 12, 14]. On kidney biopsy, C3GN (Fig. 25.1a–d) and DDD (Fig. 25.2a–c) present as a proliferative glomerulonephritis [1]. The most common pattern on light microscopy for both C3GN and DDD is that of MPGN. Other patterns of injury include mesangial proliferative glomerulonephritis, diffuse proliferative glomerulonephritis or even a necrotizing and crescentic glomerulonephritis [15]. Two or more patterns of injury may be seen on the same biopsy. On immu-

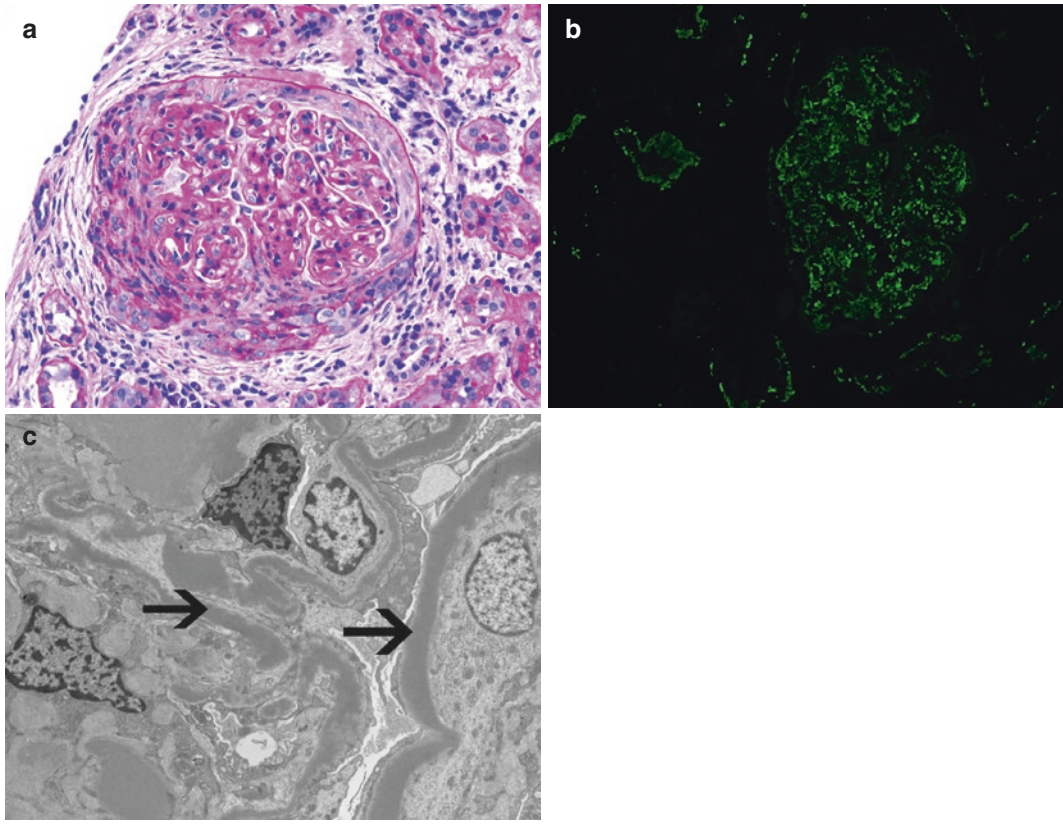

Fig. 25.2 Dense deposit disease. (**a**) Light microscopy showing an MPGN pattern of injury (periodic acid Schiff 40×). Note cellular crescent. (**b**) Immunofluorescence microscopy showing mesangial and capillary wall staining for C3. Immunofluorescence studies were negative for Ig's. (**c**) EM showing dense osmiophilic deposits along the glomerular basement membranes and in the mesangium (11,100×). *Arrows* point to the deposits

nofluorescence studies, both C3GN and DDD are characterized by bright mesangial and capillary wall staining for C3. In DDD, C3 staining may also be seen along the tubular basement membrane. Tubular basement membrane staining for C3 is uncommon in C3GN. The main differentiating factor between C3GN and DDD lies in the EM findings. In C3GN, the complement deposits are discrete and are located in the mesangium and along the capillary wall in the subendothelial region of the GBM. Subepithelial (humps) and few intramembranous deposits are also often present. The deposits often assume a lobular shape and have a waxy appearance with ill-defined margins. On the other hand, in DDD the deposits are intensely osmiophilic and are located in the mesangium and within the GBM (intramembranous deposits) often forming large dense ribbon/sausage shaped bands that can completely transform the GBM.

Lessons Learned from Proteomics in C3GN and DDD Patients

Both C3GN and DDD are diseases resulting from CAP dysregulation. Recent studies using the technique of laser microdissection of glomeruli followed by mass spectrometry showed accumulation of complement factors of the CAP including the terminal pathway and regulating proteins such as CFHR1 and CFHR5 as well as vitronectin and clusterin in both conditions [16, 17]. There was little or no significant accumulation of complement factors of the classical complement pathway, such as C1, C2 or C4. In addition, there

was little or no Ig present. Of note, there was no CFB present either, indicating absence of C3 and C5 convertase in the glomeruli, findings suggesting that CAP activation in C3G occurs in fluid phase rather than locally (as on the glomerular endothelial cells in aHUS).

Post-infectious Glomerulonephritis and C3GN

Post-infectious glomerulonephritis (PIGN) is characterized by a proliferative glomerulonephritis on light microscopy, staining for granular IgG and C3 on IF microscopy, and mesangial, subendothelial and subepithelial "hump" like deposits on EM. However, in some cases IF studies show dominant C3 with scant or no Ig staining while EM shows the characteristic "hump" like subepithelial deposits. Thus, in this setting the IF findings are similar to C3GN.

In the past, many of these cases with the "hump" like subepithelial deposits and bright C3 staining were deemed PIGN. Terms such as "resolving," "persistent" or "chronic" PIGN were used when decreased serum C3 levels, hematuria and proteinuria persisted or when there was deterioration of kidney function, as PIGN typically resolves within weeks. Recently, it was shown that cases with "hump" like subepithelial deposits, bright C3 staining and scant/no Ig and persistent decreased serum C3 levels and hematuria/proteinuria, were associated with CAP abnormalities. The term "atypical" PIGN – in analogy to aHUS – was introduced to highlight the underlying CAP abnormalities in these patients [18]. The key differentiating feature between PIGN and "atypical" PIGN is the presence of both Ig and C3 in PIGN while there is only C3 with scant or no Ig in "atypical" PIGN, even though subepithelial humps are common to both entities. It is postulated that infections activate the CAP in atypical PIGN. However, due to an underlying defect in the regulatory mechanisms, there is persistent CAP activation with resultant deposition of complement factors and ensuing inflammation in the glomeruli. Of note, subepithelial humps are also seen in DDD [19]. Thus, it is conceivable that DDD and C3GN may be triggered by an infection. However, it is the underlying regulatory CAP defect which then drives the glomerular inflammation even after the infection is controlled.

C3G Recurrence After Transplantation

DDD and C3GN have a high recurrence rate in kidney transplant recipients. For DDD, there is a 60–85 % recurrence risk, resulting in allograft failure in 45–50 % within 5 years of transplantation [20–22]. Information on C3GN recurrence is scarce. In a recent study, there was recurrence of C3GN in 66.7 % of patients, with graft loss in 33 % within 5 years [6]. Kidney biopsy of early recurrent C3GN, detected mainly on routine/protocol biopsies, shows a mesangial proliferative glomerulonephritis on light microscopy, mesangial C3 deposition on IF and mesangial electron dense deposits on EM. MPGN is more common at later stages or when the biopsy is done for clinical indications. This is similar to the findings of recurrent MPGN in general [23].

Immune (Ig/IC)-Mediated MPGN

The three main causes of glomerular Ig/IC deposition are monoclonal Ig deposition as a result of paraproteinemia, immune-complexes (IC) as result of infection, or autoimmune diseases. Monoclonal Ig is rare in the pediatric population. On the other hand, MPGN may result from chronic infections or autoimmune diseases (Table 25.1).

On LM (Fig. 25.3a–d), the glomeruli appear enlarged and show mesangial expansion with increase in cellularity. The capillary walls are thickened and show double contour formation. The capillary tufts have a distinctly lobular appearance and present with increased endocapillary cellularity. IF studies are the key to define the underlying etiology: granular polyclonal IgM, with smaller amounts of IgG along the capillary walls, with or without C3, is typical of chronic viral infections such as hepatitis C; multiple immunoglobulins, such as IgM, IgG,

Table 25.1 Secondary MPGN – synopsis

Conditions underlying secondary MPGN	Type of MPGN[a]
Infectious diseases: bacterial/viral/protozoal	
Hepatitis B, C, EBV, HIV	I, III
Endocarditis/visceral abcesses	I
Infected ventriculoatrial/ventriculoperitoneal shunts/empyema	I
Malaria, schistosomiasis, mycoplasma	I
Tuberculosis, leprosy	I, II
EBV infection	I
Brucellosis	MPGN-like pattern
Systemic immune diseases	
Cryoglobulinemia	I, III
Systemic lupus erythematosus	I, III, II
Sjögren's syndrome	III, I
Rheumatoid arthritis	I
Heriditary deficiencies of complement components	I, II
X-linked agammaglobulinemia	MPGN-like pattern
Neoplasms/dysproteinemias	
Plasma cell dyscrasia	MPGN-like pattern
Fibrillary and immunotactoid glomerulonephritis	MPGN-like pattern
Light chain deposition disease	MPGN-like pattern
Heavy chain deposition disease	MPGN-like pattern
Light and heavy chain deposition disease	MPGN-like pattern
Leukemias and lymphomas (with cryoglobulinemia)	I, III
Waldenstrom macroglobulinemia	I, III
Carcinomas, Wilms' tumor, malignant melanoma	II
Chronic liver disease	
Chronic active hepatitis (B, C)	I, III
Cirrhosis	I, III
Alpha-1-antitrypsin deficiency	I
Miscellaneous	
All conditions leading to thrombotic microangiopathy	MPGN-like pattern
Sickle cell disease	I
Partial lipodystrophy (mainly dense deposit disease)	II, I, III
Transplant glomerulopathy	MPGN-like pattern
Niemann-Pick disease (type C)	II

[a]Listed according to frequency – nomenclature based on original references

and sometimes IgA, along with C3 and C4, are noted in autoimmune diseases, such as Sjogren Syndrome, rheumatoid arthritis, etc. Lupus nephritis, with polyclonal Ig of all classes, along with C3 and C1q, may also present with an MPGN picture. EM shows glomerular capillary wall thickening with subendothelial deposits, cellular elements, and new basement membrane material resulting in double contours. Foot process effacement may be extensive. Tubuloreticular inclusions in endothelial cells point to an autoimmune etiology. The mesangium is expanded and often contains electron dense deposits which are often more sharp and discrete compared to the waxy and lobular appearance of such deposits in C3G. Deposits in small arteries and along the tubular basement membranes may be present in IC-mediated MPGN due to autoimmune diseases.

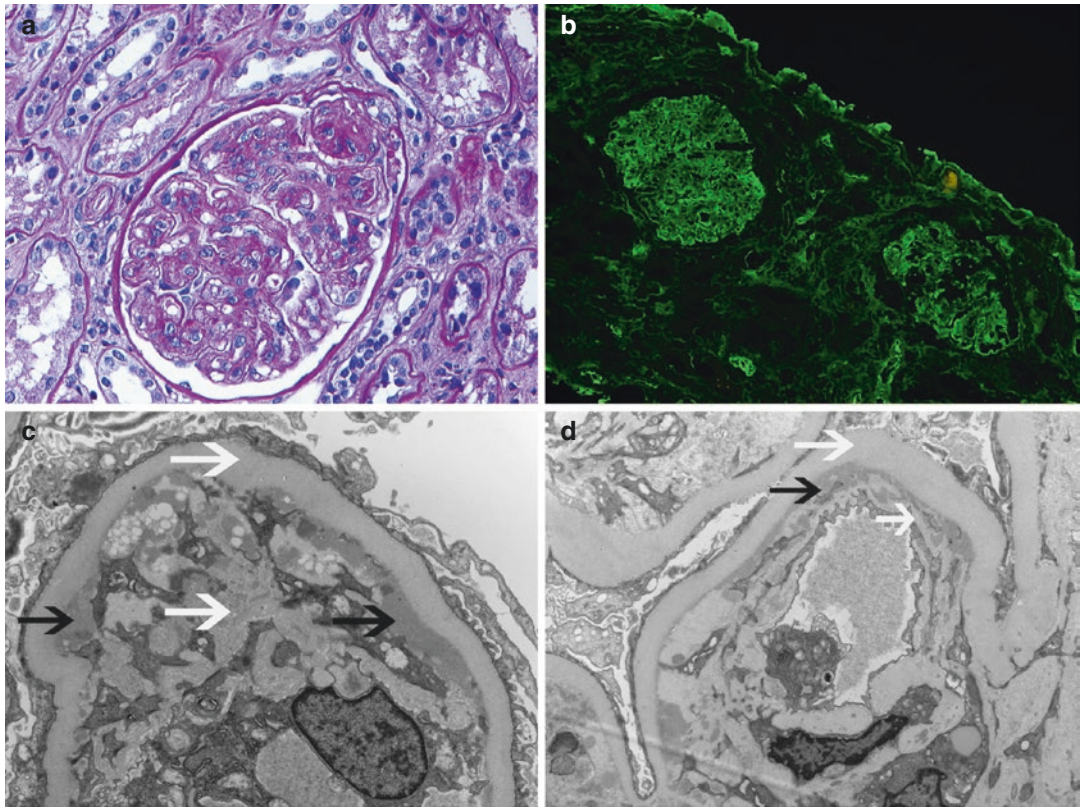

Fig. 25.3 Immune complex-mediated MPGN, in the setting of rheumatoid arthritis. (**a**) Light microscopy showing an MPGN pattern of injury (periodic acid Schiff 40×). Note lobular accentuation of glomerular tufts and thickened capillary walls. (**b**) Immunofluorescence microscopy showing mesangial and capillary wall staining for IgM (20×). (**c**, **d**) EM showing subendothelial deposits and capillary wall thickening with double conotur formation. *Black arrows* point to subdendothelial deposits, and *white arrows* point at original and new basement membrane formation (double contour) (**c**-11,100×, **d**-7,830×)

Classification Summary

The classification of C3G is based on the pattern and intensity of immunofluorescence seen on renal biopsy (Figs. 25.1, 25.2, and 25.3). Prevalent Ig staining prompts the diagnosis of a *secondary* MPGN (above), and work-up including a panel of auto-antibodies, viral serologies and, exceptionally in pediatric patients, searching for cryoglobulins and monoclonal gammopathies is warranted (Table 25.1). However, predominant C3 staining prompts the diagnosis of a primary disease, referred to as C3G. Within this category, EM assessment allows for the differentiation of patients with dense deposit disease (DDD; previously known as MPGN II), in whom dense, sausage-like very intensely osmiophilic deposits are present along the glomerular basement membrane, from patients with predominant mesangial C3 staining and possible presence of subepithelial humps as described in post-infectious mesangial glomerulonephritis. The latter group is diagnosed with MPGN I or C3GN, with MPGN I traditionally being favored in patients with membranoproliferative lesions. Of note, C3G may be familial or sporadic.

Clinical Presentation

While the clinical presentation of C3G is very heterogenous reflecting the variety of the underlying causes for CAP dysregulation, the initial manifestation of C3G is typically characterized

by (possibly nephrotic range) proteinuria, hematuria and hypertension coupled with low circulating C3 levels [24, 25]. Age of disease onset is very variable, with the earliest reported case being at age 1 [26]. In the most comprehensive clinical overview of cases [27] disease onset was below the age of 16 years in about 40 % of cases with the youngest reported age at onset of 5 years and a slight prevalence of males (60 %). Family history of glomerulonephritis was present in 11 % of cases. At onset, 41 % of patients had nephrotic-range proteinuria (>3 g/day), 61 % had microhematuria. The frequency of macrohematuria in this cohort was not reported, but was around 16 % in other reports [19]. High blood pressure was present in 30.5 % of patients. Renal function was impaired at disease onset in 45.5 % of cases, with a mean eGFR of 69.3 ml/min per 1.73 m^2. Evaluation of circulating C3 and C4 levels showed low C3 plasma levels in 46 % of all patients, more frequently in patients with DDD (60 %), in whom C3 levels were on average also lower. Low C4 was rare (only about 2 % of cases).

Disease precipitation is often associated with an infection as described in a report of children with DDD, in whom the appearance of renal symptoms was preceded by a respiratory infection in 57 % of cases [19].

Variable age at presentation is probably linked to the fact that C3G can have an indolent and remitting course, so that microhematuria and low-grade proteinuria can remain undetected for years, leading to a delayed diagnosis when proteinuria becomes nephrotic or when renal failure develops. However, early onset with nephrotic proteinuria and renal failure, though less common, has been reported [28]. Recurrent macrohematuria during banal infections is not uncommon [29, 30].

C3G can present as *nephritic syndrome*: a child with macrohematuria, glomerular microhematuria (dysmorphic red blood cells) and proteinuria of variable intensity appearing within 2–3 weeks of an infectious episode (such as upper or lower respiratory tract or gastroenteritis). Laboratory exams usually reveal low circulating C3 with normal C4 and a variable degree of renal impairment. Hypertension may be present. This picture is (clinically) compatible with post-infectious glomerulonephritis (PIGN), IgA nephropathy (if C3 is normal), or (especially in adolescent females with decreased C4) lupus nephritis.

C3G can also present as *nephrotic syndrome*: patients present with peripheral edema (and weight gain), abdominal pain, and in some cases reduced urine output and usually marked hypertension. Laboratory exams show nephrotic-range proteinuria (>40 mg/h/m^2 or urinary protein/creatinine >2 mg/mg, respectively) with microhematuria, typically some degree of (acute) renal failure, hypoalbuminemia, elevated cholesterol, triglycerides and platelets, and low immunoglobulins. C3 levels are typically low. This presentation can lead to treatment with steroids, and only subsequently, in the absence of response to this treatment, be deemed an indication for a renal biopsy.

In other cases, C3G presents with *microhematuria and low-grade proteinuria* only. In this case examination of the patient will be unremarkable, and laboratory evaluations may be normal except for low circulating C3 (with normal C4) levels.

In general, a family history of glomerulonephritis must be investigated, as familial forms of C3G are described and genetic investigations may be channeled more effectively in these cases. In addition, establishing the diagnosis of C3G not only warrants a renal biopsy (histopathological findings described in detail above) but also detailed work-up of the complement system including (Table 25.2) [10, 14]:

1. Complement factors (CFH; CFB)
2. CAP activation markers (C3; C4; APH50; CH50; C3d; C5a; SC5b9)
3. Circulating autoantibodies (C3 Nephritic Factor (C3NeF), anti-CFH autoantibodies [31] and anti-CFB autoantibodies [32])
4. Mutations in factors involved in CAP regulation (Factor H [CFH]; Factor I [CFI]; membrane cofactor protein [MCP/CD46]; C3 and Factor B [CFB])
5. Copy number variations and hybrid gene formation within the complement CFH related (CFHR) gene locus, in particular internal duplications within *CFHR5* [33, 34], *CFHR3-1* [35], or other rearrangements (reviewed in [10]).

Servais et al. identified mutations in complement genes in 18 % of patients, while the presence

Table 25.2 Diagnostic evaluation of a patient with suspected diagnosis of C3G: *complement system*

Complement factors	Complement activation markers	Antibodies	Mutations	Copy number variations and hybrid genes
CFH	C3	C3NeF	CFH	CFHR3-1
CFI	C3d	Anti-CFH	Cfi	CFHR5
	C4	Anti-CFB	MCP/CD46	
	C5a		C3	
	C5b-9		CFB	
	APH50			
	CH50			

of circulating C3Nef was detected in 59% of cases [13]. C3Nef is an autoantibody capable of binding the CAP C3 convertase, C3BbB, thus conferring resistance to its inactivation by regulatory factors such as CFH.

Of note, more than half of the patients carrying complement gene mutations were also C3NeF positive. C3Nef was more frequent in patients with DDD (86%) and was associated with significantly lower levels of circulating C3. A report of three patients with DDD showed a correlation of moderate increases in C3Nef and slight reduction on C3 with disease recurrence post-transplant [36]. In other reports, lower circulating levels of C3 were found in patients with a membranoproliferative pattern of disease [28], while others have reported that children have significantly lower C3 levels and more frequent C3Nef positivity compared to adults [19]. C3NeF can be detected in different ways [37, 38]. Some assays use patient purified immunoglobulins to screen for autoantibodies that stabilize C3bBb [38], others infer the presence of C3bBb-stabilizing autoantibodies by detection of C3 breakdown products [38]. It is possible that patients may be positive in some but not all of these assays, and C3Nef levels have been found occasionally also in healthy individuals [39]. Therefore, the significance of C3Nef in the pathophysiology of C3G and its correlation with disease course and treatment response is still debated and warrants further investigation [10, 40].

Symptoms

C3G is a complement-mediated disease, secondary to CAP dysregulation. Given that this dysregulation occurs in the fluid phase of blood, extra-renal features of disease are to be expected. In DDD, patients may develop acquired partial lipodystrophy (APL) [39, 41, 42] and ocular lesions similar to soft drusen seen in age-related macular degeneration (AMD) [43]. Moreover, the presence of C3 deposits has also been described in the spleen of patients with DDD [44].

Acquired partial lipodystrophy – like DDD and C3GN – is associated with dysregulation of CAP on adipocytes and becomes manifest in the loss of subcutaneous fat tissue, which typically occurs in the upper half of the body (starting from the face and extending to involve the neck, shoulders, arms and thorax) and precedes the onset of renal disease by several years. Median interval between the onset of APL and DDD is about 8 years [45]. The majority of APL patients presents with low C3 levels and are C3NeF positive. Patients with combined disease are more likely to present with decreased C3 levels and develop APL earlier in life (about 7.7 years of age) [45]. A common cause – unrestricted CAP activation – for both APL and DDD is suggested, and complement mediated destruction of adipocytes has been shown [39, 42].

Patients with DDD can also develop ocular lesions in the form of drusen. Drusen are retinal changes seen as crystalline yellow or white dots, which lie between the retinal pigment epithelium and Bruch's membrane [46]. Drusen can develop in the second decade of life and are responsible for visual disturbances in up to 10% of DDD patients [43]. The drusen seen in DDD patients are similar to those in age-related macular degeneration (AMD), which represents the major cause of blindness in the Western aging population. In the

early phase of AMD, drusen can develop without any visual problems (i.e., soft drusen), but can progress to visual loss after 65 years of age [47, 48]. Genome scan studies have linked AMD to the "regulators of complement" (RCA) gene cluster on chromosome 1q32 [49]. Moreover, recently a single-nucleotide *CFH* polymorphism (Y402H) was found to be crucial in the development of AMD [49–52]. Studies of the composition of drusen support this link by confirming the presence of CFH in drusen of AMD patients [46, 49].

Outcome and Risk of Relapse Post-transplantation

The natural history of C3G is still quite obscure, but available data do not indicate a favorable outcome. Fifty percent of DDD patients with disease for 10 years or more progress to end-stage renal disease (ESRD), with young girls having the greatest risk for renal failure [22]. Forty-five percent of renal allografts are lost within 5 years of transplant [22]. Concerning risk of relapse following renal transplantation for DDD, in one study the degree of proteinuria was strongly associated with disease recurrence, and the presence of glomerular crescents in biopsies of renal allografts had a significant negative correlation with graft survival [21]. However, a correlation with the severity of hypocomplementemia either at initial presentation or at the time of disease recurrence in the renal allograft was not found [21].

In reports evaluating C3G [27, 28], at last follow-up about 37 % of patients were on dialysis. Median time from first observation to ESRD was about 10 years, and in the patients that underwent renal transplantation, disease recurrence was observed in >50 % of cases, with an additional 17 % experiencing thrombotic microangiopathy (TMA). In the familial form of C3G secondary to CFHR5 mutations described in individuals of Cypriot descent [33] there was a significant difference in prognosis between sexes: among mutation carriers, men were by far more likely to progress to chronic kidney disease (CKD) and ESRD than women (78 % vs 22 %).

Currently, the available data on C3G are too limited to correlate findings on genetic, autoantibody, and complement function screening with prognosis or progression of disease [53]. As the number of identified cases increases, it is likely that some of these pathogenetic parameters will be found to be biomarkers of disease progression. In small case series, however, the best available predictors for disease outcome remain the standard clinical parameters, such as degree of renal dysfunction, measured by serum creatinine or estimated glomerular filtration rate (eGFR), proteinuria and blood pressure at the time of diagnosis [43]. In one series of patients with DDD, older age at diagnosis also emerged as an independent predictor of ESRD in a multivariate analysis [19]. Others have suggested that patients with C3GN may have a more benign course than patients with DDD, indicating differences in the degree or nature of CAP activation that correlate with the differences in histology [1, 12, 27]. Thus, the formal assessment of a newly diagnosed C3G must consider traditional predictors of renal outcomes, particularly when trying to gauge prognosis and evaluate the need for therapy [53].

Differential Diagnosis

The presentation of a nephritic/nephrotic clinical picture is compatible with a variety of diagnoses at disease onset (Table 25.3). The clinical picture can be indistinguishable from IgA nephropathy (IgAN), particularly if the circulating C3 is normal and macrohematuria is observed [29]. The presence of familial disease does not exclude the diagnosis of C3G, particularly but not exclusively in patients of Cypriot descent [33, 35]. Renal biopsy, in particular the immunofluorescence, allows clear discrimination between C3G and IgAN.

In the presence of reduced C3 levels, particularly in context of a recent infection, the diagnosis of acute post-infectious glomerulonephritis (PIGN) needs to be considered. In this case, a renal biopsy may not be definitive as the presence of "humps" in the EM is common to both, C3G and PIGN [54]. The latter presents with reduced circulating C3 levels, which typically normalize within 1–3 months from disease onset. As C3 levels may be normal also in C3G and given the heterogeneity of the clinical picture of this disease

Table 25.3 Diagnostic evaluation of a patient with suspected diagnosis of C3G: *non-complement aspects*

History	Family history of hematuria, proteinuria, renal failure
	Macrohematuria (if yes, concomitant to infection?)
	Infection (especially URTI) in the preceding weeks
	Reduction in urine output, frothy urine
	Symptoms of systemic disease (e.g., weight loss, fever, arthralgia, rash, petechiae)
Clinical examination	Signs of nephrotic syndrome (peripheral edema; bloodwork)
	Blood pressure
	Urine dipstick
	Ocular examination (drusen)
	Partial lipodystrophy
Laboratory work-up	Urinalysis, spot urine or 24-h PCR and ACR
	Complete blood count, urea, creatinine, protein, IgG, IgA, IgM
	Serum C3, C4
	Auto-antibodies (ANA, anti-dsDNA, ANCA)
	ASOT
	Renal ultrasound

entity, we suggest that in the case of a clinical picture common to C3G and PIGN, even with normalizing C3 levels and absent proteinuria, patients be advised to perform urinalysis e.g., every 3–6 months for 2 years following resolution of the acute clinical picture and to seek medical attention if macrohematuria or significant proteinuria (>20 mg/dl) re-appear.

Management

At present, there is no treatment standard or therapeutic agent of proven effectiveness in C3G available. The rarity of this disease, coupled with its protracted and variable natural history, make clinical trials logistically challenging. Moreover, performing a literature review has to consider the fact that with advanced pathogenetic insights the nomenclature in the field has changed, and literature specifically referring to C3G is consequently very scarce. However, considering that about 50 % of patients proceed to ESRD and may face a high risk of disease recurrence post-renal transplant, concerted efforts to define effective treatment strategies are necessary.

Searches performed on the existing literature using the terms "dense deposit disease" and "idiopathic membranoproliferative glomerulonephritis" show that randomized clinical trials are very few, and the use of different end-points make uniform interpretation of results difficult [55]. Several therapeutic regimens have been employed, utilizing immunosuppressive agents (glucocorticoids, mycophenolate mofetil, calcineurin inhibitors), anti-platelet agents, plasma exchange or infusion and, much more recently, complement blockers [56, 57]. Renoprotective agents (such as angiotensin-converting-enzyme inhibitors, ACEI, or angiotensin II receptor antagonists, ARB) are associated with these treatments almost invariably. As our understanding of the pathophysiology of C3G is rapidly expanding and changing, while reviewing published cases is reasonable, its usefulness in guiding future therapeutic strategies may be limited [10].

The therapeutic strategy should be driven by clinical parameters, such as the degree of proteinuria and impairment in renal function, and also by diagnostic test results. In the near future, the availability of new therapeutic agents may drastically alter this strategy. Clinicians need to be aware that clinical practices in this field may evolve rapidly.

In the following paragraphs regarding treatment, for all options except eculizumab the literature cited is about different forms of idiopathic or primary MPGN, including DDD.

Immunosuppressive Agents

Prednisone

There are no published trials on the use of prednisone in C3G. Existing literature pertains to primary MPGN of all subtypes. In children with primary MPGN, prednisone – specifically, long-term low-dose use of prednisone – was found to have a beneficial effect with respect to the degree of proteinuria and renal survival [58–61]. This observation was confirmed by subsequent studies, in which therapy

with prolonged alternate day prednisone delayed deterioration of renal function [60, 62].

However, response of MPGN patients to corticosteroids is not homogenous. A MPGN subtype specific analysis of the effect of corticosteroid treatment revealed a lack of efficacy in patients with MPGN II/DDD, despite a beneficial effect on all MPGN patients irrespective of the MPGN subtype [60].

Altogether, in forms of C3G other than DDD, glucocorticoids may be effective, but their nonspecific nature and adverse effects mean that a high price is paid for any beneficial effect on the renal lesion [63]. A reasonable approach based on current knowledge may be that of utilizing 40 mg/m^2 alternate-day prednisone for 6–12 months in patients with a C3G that presents with nephrotic-range proteinuria, with or without renal failure. If no significant reduction of proteinuria is observed, steroids should be tapered and discontinued [64]. It is important to recognize that a number of patients with C3G will not respond to this therapeutic approach [10].

Other Immunosuppressive Agents

In idiopathic MPGN patients, MMF was administered alone or in combination with corticosteroids, and generated encouraging results [65]. Another report of 13 adult patients with idiopathic MPGN resistant to glucocorticoid treatment (8 weeks at 1 mg/kg/day) showed that adding MMF led to significant reduction of proteinuria and increase of eGFR [66]. MMF in addition to pulse and long-term steroid treatment was also found effective in a pediatric patient [67]. No published reports on the effectiveness of MMF in DDD are available. In a recently published metaanalysis of 60 patients with C3GN with a median follow-up of 47 months, 22 patients were treated with a combination of corticosteroids and MMF. Compared to patients with no treatment or other immunosuppressive regimens, patients treated with corticosteroids and MMF showed the best outcome with respect to disease progression (i.e. decline in kidney function) and renal survival.

Calcineurin inhibitors (i.e., cyclosporine and tacrolimus) are also used in the treatment of MPGN. The efficacy of cyclosporine was recently tested with encouraging results in a trial involving 18 patients with refractory MPGN who also received small doses of prednisolone (0.15 mg/kg/day). Long-term reductions in proteinuria with preservation of renal function was observed in 17 of the patients [68]. In two children with idiopathic MPGN with suboptimal response to a prolonged course of steroids, rapid and complete remission of the nephrotic syndrome was achieved after initiation of tacrolimus [69].

Contradictory results are published about the efficacy of cyclosporine A in the treatment of patients with MPGN II/DDD. While Kiyomasu et al. report recovery from nephrotic syndrome using a combination of alternate-day low-dose prednisone and cyclosporine [70], a beneficial effect of calcineurin inhibitors was not seen in other patients [43].

The use of rituximab has been suggested in the presence of circulating C3NeF. However, to the best of our knowledge, while there are no published results showing this treatment to be effective, there are a few single case reports of its inefficacy [71, 72].

Altogether, limited uncontrolled data suggest that MMF or CNI may be of use in patients with C3G and high-grade proteinuria resistant to glucocorticoids and at present there is insufficient evidence to support the use of cyclophosphamide or rituximab in children with this disease [64].

Plasma Infusion or Plasma Exchange

As reviewed by Smith et al. [40] in CFH-deficient mice with C3G, renal C3 deposition and its depletion in plasma are rapidly reversed when (either mouse or human) CFH is administered [73, 74]. These data are in keeping with observations made in the first naturally occurring animal model of DDD in Norwegian pigs, in which a truncating *Cfh* mutation in SCR15 resulted in the absence of CFH from plasma [75–77]. Treatment with purified porcine or human CFH delayed onset of the DDD phenotype and progression to ESRD significantly [76, 77]. These outcomes suggest that in some C3G patients with CFH mutations, CFH

replacement therapy could restore the underlying defect and correct the disease. This has been shown in two siblings with DDD secondary to a functional CFH defect in whom chronic plasma infusion prevented disease progression and development of ESRD [30, 78] as well as in a MPGN I patient with a MCP/CD46 mutation refractory to conventional treatments who also responded favorably to chronic plasma infusion [79].

Purified CFH preparations may be available for therapeutic use in the future [80, 81]. Whether administration of exogenous CFH to patients without *CFH* mutations would be therapeutically successful is unclear; however, patients with C3 mutations rendering the C3 convertase resistant to regulation by CFH, are expected to be refractory to treatment with CFH [82].

In all scenarios characterized by deficiency or functional defect of one or more complement components, replacement of this factor/these factors by either plasma infusion or plasma exchange could theoretically be effective [83]. For plasma infusion, volumes of 10–20 ml/kg/treatment (fresh frozen, solvent-detergent or cryosupernatant plasma), and treatment intervals of 14 days based on the measured CFH half life of about 6 days [84], seemed to be adequate [30, 78].

Plasma exchange allows removal of either dysfunctional endogenous complement factors, which – in addition to their functional impairment – might also compete for potential binding partners/receptors, thus possibly weakening the efficacy of plasma replacement therapy. Furthermore, plasma exchange removes antibodies like the IgG autoantibody C3NeF and CFH/CFB autoantibodies. Plasmapheresis/plasma exchange has been reported to be beneficial in MPGN I [85], and MPGN II/DDD [21, 86]. Conversely, McCaughan et al. reported an inability to establish remission in DDD despite the documented removal of C3Nef via plasmapheresis [72].

Because of discordant reports in the literature and the absence of definitive therapy, it is likely that plasma therapy will continue to be used on a case-by-case basis in C3G [10]. However, this therapy may be attempted, in the absence of response to immunosuppression, in rapidly progressive forms, particularly if defective CFH or anti-CFH autoantibodies are found.

Complement Inhibitors

Our advanced understanding of C3G pathogenesis suggests therapeutic targeting of CAP dysregulation by complement inhibition. Based on the pathophysiology of disease, anticomplement therapy warrants consideration. This could include (1) C3 convertase inhibition, which may have its greatest utility in limiting C3 breakdown product deposition on (glomerular) basement membranes; (2) C5 or terminal complement pathway inhibition [10].

While somewhat conflicting with *in vivo* observations, in *cfh*,*cfi* double knock out mice, which identified a critical role for uncontrolled C3 conversion in MPGN (vs. uncontrolled C5 conversion in aHUS) [87], effectiveness of anti-C5 therapy in a mouse model of DDD (*Cfh* deficient mice) [88] provided the rationale and led to the use of a humanized anti-C5 monoclonal antibody (eculizumab) in patients with different forms of C3G [71, 72, 79, 89–91].

Eculizumab is a humanized monoclonal antibody directed against C5, which blocks C5 cleavage, thus preventing the release of C5a, a potent anaphylatoxin, and C5b, the initial protein of the cytotoxic membrane attack complex (MAC; C5b-9). Its use has been recently approved for aHUS, the classic model of renal disease mediated by the CAP. Up to now its use has been reported in 11 C3G patients, including six cases of C3G affecting the native kidneys [71, 79, 89, 91] and five cases of C3G recurring in the renal graft [72, 90, 91]. The results from these studies are encouraging in 8/11 patients, in whom treatment with eculizumab led to some improvement in renal parameters (proteinuria and/or renal function and/or histology). Nonetheless, response in most cases appears to be less transformative than what has been reported so far concerning the use of eculizumab in patients with aHUS. Interestingly, an increased level of circulating SC5b-9 tended to correlate with a beneficial effect of eculizumab on the course of C3G. The other most relevant factor

in predicting response to eculizumab appears to be disease duration, which was longer in non-responders. The limited available pathological data indicate that eculizumab decreased endocapillary proliferation and inflammatory cell infiltration in four out of six patients who underwent repeat kidney biopsy [89, 92]. Taken together, while encouraging, the limited data available does not allow for proposing eculizumab as standard therapy for C3G yet, and a prospective treatment trial will be needed to answer this question.

Recently, a report was published on the effectiveness of Tripterygium wilfordii (TW or triptolide), a herbal extract shown to have in vitro immunomodulatory effects and the ability to reduce renal complement expression [93], in reducing proteinuria in different degrees in eight out of ten patients with DDD [94]. The broader therapeutic use of this option, that has been employed for rheumatoid arthritis, is limited by reports of severe side effects in about half of treated patients [43].

A potential benefit of complement inhibition in the treatment of complement-mediated diseases needs to be balanced against the detrimental effect of complement inhibition in situations when complement activation is required as part of the physiological immune defense of the host, and clinical trials are required before the use of these novel substances in children can be recommended.

Renoprotective Agents

About 80 % of C3G patients are placed on ACEIs or ARBs as first line agents used to improve renal dynamics, decrease proteinuria, control blood pressure and limit glomerular leukocyte infiltration [40]. This approach is recommended as exclusive treatment in two cases:

1. Non-nephrotic proteinuria with or without microhematuria and normal renal function/absence of acute renal failure. Close follow-up is needed to assess progression of disease based on renal function, proteinuria, and urine microscopy.
2. Histological evidence of advanced chronicity of the renal lesions on the biopsy.
   Patients with advanced CKD, severe tubulointerstitial fibrosis (TIF), or other findings consistent with chronic disease sequel should – in the absence of systemic disease manifestations – not be treated with immunosuppression [64].

Treatment of Recurrence Post-renal Transplantation

There is no proven beneficial therapy for recurrent C3G in the renal allograft following transplantation. Therapeutic approaches are similar to those used in primary disease manifestation and are therefore not discussed in detail here.

Reported treatment of *recurrent idiopathic MPGN*, besides conservative medications, include antiplatelet/anticoagulant agents [95], corticosteroids [96], cyclosporine [97], cyclophosphamide [98], and plasmapheresis [96, 99]. Reported treatments of *recurrent DDD* include dose reduction, discontinuation or switch (cyclosporine to tacrolimus) of the CNI used as part of the posttransplant immunosuppression regimen, modification of the prednisone dose (increase; switch from daily to alternate-day), pulse methylprednisolone, or plasmapheresis/plasma exchange [21, 86].

In summary, the therapeutic options in C3G depend on the level of proteinuria and kidney failure and on the results of the diagnostic tests performed. In the vast majority of patients, the empiric use of ACEI or ARB as drugs of first choice to treat hypertension and decrease proteinuria is a common practice, which may delay the progression of renal disease [63]. Plasma therapy or, in the future, purified CFH may be useful in some cases in which there is clear evidence of a CFH deficiency or of the presence of anti-CFH autoantibodies. There is little evidence of the effectiveness of immunosuppression, which should therefore be employed only in cases where disease is very active and proliferative with intense inflammation in the renal biopsy and nephrotic-range proteinuria. Preliminary results on the use of complement

blockers such as eculizumab are encouraging in some but not in all patients.

References

1. Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis: a new look at an old entity. N Engl J Med. 2012;366:1119–31.
2. Sethi S. Etiology-based diagnostic approach to proliferative glomerulonephritis. Am J Kidney Dis. 2014;63:561–6.
3. Nasr SH, Satoskar A, Markowitz GS, Valeri AM, Appel GB, Stokes MB, Nadasdy T, D'Agati VD. Proliferative glomerulonephritis with monoclonal IgG deposits. J Am Soc Nephrol. 2009;20: 2055–64.
4. Sethi S, Zand L, Leung N, Smith RJH, Jevremonic D, Herrmann SS, Fervenza FC. Membranoproliferative glomerulonephritis secondary to monoclonal gammopathy. Clin J Am Soc Nephrol. 2010;5:770–82.
5. Sethi S, Rajkumar SV. Monoclonal gammopathy – associated proliferative glomerulonephritis. Mayo Clin Proc. 2013;88:1284–93.
6. Zand L, Lorenz EC, Cosio FG, Fervenza FC, Nasr SH, Gandhi MJ, Smith RJ, Sethi S. Clinical findings, pathology, and outcomes of C3GN after kidney transplantation. J Am Soc Nephrol. 2014;25(5):1110–7.
7. Rennke HG. Secondary membranoproliferative glomerulonephritis. Kidney Int. 1995;47:643–56.
8. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB, Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi L-M, Makino H, Moura LA, Nagata M. The classification of glomerulonephritis in systemic lupus erythematosus revisited. J Am Soc Nephrol. 2004;15:241–50.
9. Zand L, Fervenza FC, Nasr SH, Sethi S. Membranoproliferative glomerulonephritis associated with autoimmune diseases. J Nephrol. 2014;27(2):165–71.
10. 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 L-H, Peters DK, Rodriguez de Cordoba S, Servais A, Sethi S, Song W-C, Tamburini P, Thurman JM, Zavros M, Cook HT. C3 glomerulopathy: consensus report. Kidney Int. 2013;84(6):1079–89.
11. Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis: pathogenetic heterogeneity and proposal for a new classification. Semin Nephrol. 2011;31:341–8.
12. Sethi S, Nester CM, Smith RJH. Membranoproliferative glomerulonephritis and C3 glomerulopathy: resolving the confusion. Kidney Int. 2012;81:434–41.
13. Servais A, Noel L, Fremeaux-Bacch IV, Lesavre P. C3 glomerulopathy. Contrib Nephrol. 2013;181:185–93.
14. Fakhouri F, Fremeaux-Bacchi V, Noel L-H, Cook HT, Pickering MC. C3 glomerulopathy: a new classification. Nat Rev Nephrol. 2010;6:494–9.
15. Fervenza FC, Smith RJH, Sethi S. Association of a novel complement factor H mutation with severe crescentic and necrotizing glomerulonephritis. Am J Kidney Dis. 2012;60:126–32.
16. Sethi S, Vrana JA, Theis JD, Dogan A. Mass spectrometry based proteomics in the diagnosis of kidney disease. Curr Opin Nephrol Hypertens. 2013;22:273–80.
17. Sethi S, Gamez JD, Vrana JA, Theis JD, Bergen III HR, Zipfel PF, Dogan A, Smith RJH. Glomeruli of dense deposit disease contain components of the alternative and terminal complement pathway. Kidney Int. 2009;75:952–60.
18. Sethi S, Fervenza FC, Zhang Y, Zand L, Meyer NC, Borsa N, Nasr SH, Smith RJH. Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement. Kidney Int. 2013;83:293–9.
19. Nasr SH, Valeri AM, Appel GB, Sherwinter J, Stokes MB, Said SM, Markowitz GS, D'Agati VD. Dense deposit disease: clinicopathologic study of 32 pediatric and adult patients. Clin J Am Soc Nephrol. 2009;4:22–32.
20. Andresdottir MB, Assmann KJ, Hoitsma AJ, Koene RA, Wetzels JF. Renal transplantation in patients with dense deposit disease: morphological characteristics of recurrent disease and clinical outcome. Nephrol Dial Transplant. 1999;14:1723–31.
21. Braun MC, Stablein DM, Hamiwka LA, Bell L, Bartosh SM, Strife CF. Recurrence of membranoproliferative glomerulonephritis type II in renal allografts: the North American Pediatric Renal Transplant Cooperative Study experience. J Am Soc Nephrol. 2005;16:2225–33.
22. Lu D-F, Moon M, Lanning L, McCarthy A, Smith RH. Clinical features and outcomes of 98 children and adults with dense deposit disease. Pediatr Nephrol. 2012;27:773–81.
23. Lorenz EC, Sethi S, Leung N, Dispenzieri A, Fervenza FC, Cosio FG. Recurrent membranoproliferative glomerulonephritis after kidney transplantation. Kidney Int. 2010;77:721–8.
24. Schwertz R, Rother U, Anders D, Gretz N, Scharer K, Kirschfink M. Complement analysis in children with idiopathic membranoproliferative glomerulonephritis: a long-term follow-up. Pediatr Allergy Immunol. 2001;12:166–72.
25. Schwertz R, de Jong R, Gretz N, Kirschfink M, Anders D, Scharer K. Outcome of idiopathic membranoproliferative glomerulonephritis in children. Arbeitsgemeinschaft Padiatrische Nephrologie. Acta Paediatr. 1996;85:308–12.
26. Servais A, Noel LH, Dragon-Durey MA, Gubler MC, Remy P, Buob D, Cordonnier C, Makdassi R, Jaber W, Boulanger E, Lesavre P, Fremeaux-Bacchi V. Heterogeneous pattern of renal disease associated

with homozygous factor H deficiency. Hum Pathol. 2001;42:1305–11.
27. 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. Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. Kidney Int. 2012;82:454–64.
28. Servais A, Fremeaux-Bacchi V, Lequintrec M, Salomon R, Blouin J, Knebelmann B, Grunfeld JP, Lesavre P, Noel LH, Fakhouri F. Primary glomerulonephritis with isolated C3 deposits: a new entity which shares common genetic risk factors with haemolytic uraemic syndrome. J Med Genet. 2007;44:193–9.
29. Karumanchi SA, Thadhani R. A complement to kidney disease: CFHR5 nephropathy. Lancet. 2010;376: 748–50.
30. Habbig S, Mihatsch MJ, Heinen S, Beck B, Emmel M, Skerka C, Kirschfink M, Hoppe B, Zipfel PF, Licht C. C3 deposition glomerulopathy due to a functional factor H defect. Kidney Int. 2009;75:1230–4.
31. Jokiranta TS, Solomon A, Pangburn MK, Zipfel PF, Meri S. Nephritogenic lambda light chain dimer: a unique human miniautoantibody against complement factor H. J Immunol. 1999;163:4590–6.
32. Strobel S, Zimmering M, Papp K, Prechl J, Jozsi M. Anti-factor B autoantibody in dense deposit disease. Mol Immunol. 2010;47:1476–83.
33. Gale DP, de Jorge EG, Cook HT, Martinez-Barricarte R, Hadjisavvas A, McLean AG, Pusey CD, Pierides A, Kyriacou K, Athanasiou Y, Voskarides K, Deltas C, Palmer A, Fremeaux-Bacchi V, de Cordoba SR, Maxwell PH, Pickering MC. Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis. Lancet. 2010;376:794–801.
34. Medjeral-Thomas N, Malik TH, Patel MP, Toth T, Cook HT, Tomson C, Pickering MC. A novel CFHR5 fusion protein causes C3 glomerulopathy in a family without Cypriot ancestry. Kidney Int. 2014;85: 933–7.
35. Malik TH, Lavin PJ, Goicoechea de Jorge E, Vernon KA, Rose KL, Patel MP, de Leeuw M, Neary JJ, Conlon PJ, Winn MP, Pickering MC. A hybrid CFHR3-1 gene causes familial C3 glomerulopathy. J Am Soc Nephrol. 2012;23:1155–60.
36. West CD, Bissler JJ. Nephritic factor and recurrence in the renal transplant of membranoproliferative glomerulonephritis type II. Pediatr Nephrol. 2008;23: 1867–76.
37. Paixao-Cavalcante D, Lopez-Trascasa M, Skattum L, Giclas PC, Goodship TH, de Cordoba SR, Truedsson L, Morgan BP, Harris CL. Sensitive and specific assays for C3 nephritic factors clarify mechanisms underlying complement dysregulation. Kidney Int. 2012;82:1084–92.
38. Zhang Y, Meyer NC, Wang K, Nishimura C, Frees K, Jones M, Katz LM, Sethi S, Smith RJ. Causes of alternative pathway dysregulation in dense deposit disease. Clin J Am Soc Nephrol. 2012;7:265–74.
39. Mathieson PW, Wurzner R, Oliveria DB, Lachmann PJ, Peters DK. Complement-mediated adipocyte lysis by nephritic factor sera. J Exp Med. 1993;177:1827–31.
40. Smith RJ, Harris CL, Pickering MC. Dense deposit disease. Mol Immunol. 2011;48:1604–10.
41. Meri S. Loss of self-control in the complement system and innate autoreactivity. Ann N Y Acad Sci. 2007;1109:93–105.
42. Mathieson PW, Peters DK. Lipodystrophy in MCGN type II: the clue to links between the adipocyte and the complement system. Nephrol Dial Transplant. 1997; 12:1804–6.
43. Appel GB, Cook HT, Hageman G, Jennette JC, Kashgarian M, Kirschfink M, Lambris JD, Lanning L, Lutz HU, Meri S, Rose NR, Salant DJ, Sethi S, Smith RJ, Smoyer W, Tully HF, Tully SP, Walker P, Welsh M, Wurzner R, Zipfel PF. Membranoproliferative glomerulonephritis type II (dense deposit disease): an update. J Am Soc Nephrol. 2005;6:1392–403.
44. Thorner P, Baumal R. Extraglomerular dense deposits in dense deposit disease. Arch Pathol Lab Med. 1982;106:628–31.
45. Misra A, Peethambaram A, Garg A. Clinical features and metabolic and autoimmune derangements in acquired partial lipodystrophy: report of 35 cases and review of the literature. Medicine (Baltimore). 2004;83:18–34.
46. de Jong PT. Age-related macular degeneration. N Engl J Med. 2006;355:1474–85.
47. Hogg RE, Chakravarthy U. Visual function and dysfunction in early and late age-related maculopathy. Prog Retin Eye Res. 2006;25:249–76.
48. Magnusson KP, Duan S, Sigurdsson H, Petursson H, Yang Z, Zhao Y, Bernstein PS, Ge J, Jonasson F, Stefansson E, Helgadottir G, Zabriskie NA, Jonsson T, Bjornsson A, Thorlacius T, Jonsson PV, Thorleifsson G, Kong A, Stefansson H, Zhang K, Stefansson K, Gulcher JR. CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. PLoS Med. 2006;3:e5.
49. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ, Hardisty LI, Hageman JL, Stockman HA, Borchardt JD, Gehrs KM, Smith RJ, Silvestri G, Russell SR, Klaver CC, Barbazetto I, Chang S, Yannuzzi LA, Barile GR, Merriam JC, Smith RT, Olsh AK, Bergeron J, Zernant J, Merriam JE, Gold B, Dean M, Allikmets R. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci U S A. 2005;102: 7227–32.
50. Edwards AO, Ritter 3rd R, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005;308:421–4.
51. Haines JL, Hauser MA, Schmidt S, Scott WK, Olson LM, Gallins P, Spencer KL, Kwan SY, Noureddine M, Gilbert JR, Schnetz-Boutaud N, Agarwal A, Postel

EA, Pericak-Vance MA. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005;308:419–21.
52. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, Haynes C, Henning AK, SanGiovanni JP, Mane SM, Mayne ST, Bracken MB, Ferris FL, Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. Science. 2005;308:385–9.
53. Bomback AS, Appel GB. Pathogenesis of the C3 glomerulopathies and reclassification of MPGN. Nat Rev Nephrol. 2012;8:634–42.
54. Sethi S, Fervenza FC, Zhang Y, Zand L, Vrana JA, Nasr SH, Theis JD, Dogan A, Smith RJ. C3 glomerulonephritis: clinicopathological findings, complement abnormalities, glomerular proteomic profile, treatment, and follow-up. Kidney Int. 2012;82:465–73.
55. Noris M, Remuzzi G. Translational mini-review series on complement factor H: therapies of renal diseases associated with complement factor H abnormalities: atypical haemolytic uraemic syndrome and membranoproliferative glomerulonephritis. Clin Exp Immunol. 2008;151:199–209.
56. Nester CM, Smith RJ. Diagnosis and treatment of C3 glomerulopathy. Clin Nephrol. 2013;80:395–403.
57. Nester CM, Smith RJ. Treatment options for C3 glomerulopathy. Curr Opin Nephrol Hypertens. 2013;22:231–7.
58. McEnery PT. Membranoproliferative glomerulonephritis: the Cincinnati experience – cumulative renal survival from 1957 to 1989. J Pediatr. 1990;116:S109–14.
59. West CD. Childhood membranoproliferative glomerulonephritis: an approach to management. Kidney Int. 1986;29:1077–93.
60. Tarshish P, Bernstein J, Tobin JN, Edelmann Jr CM. Treatment of mesangiocapillary glomerulonephritis with alternate-day prednisone – a report of the International Study of Kidney Disease in Children. Pediatr Nephrol. 1992;6:123–30.
61. Ford DM, Briscoe DM, Shanley PF, Lum GM. Childhood membranoproliferative glomerulonephritis type I: limited steroid therapy. Kidney Int. 1992;41:1606–12.
62. Yanagihara T, Hayakawa M, Yoshida J, Tsuchiya M, Morita T, Murakami M, Fukunaga Y. Long-term follow-up of diffuse membranoproliferative glomerulonephritis type I. Pediatr Nephrol. 2005;20:585–90.
63. Alchi B, Jayne D. Membranoproliferative glomerulonephritis. Pediatr Nephrol. 2010;25:1409–18.
64. Beck L, Bomback AS, Choi MJ, Holzman LB, Langford C, Mariani LH, Somers MJ, Trachtman H, Waldman M. KDOQI US commentary on the 2012 KDIGO clinical practice guideline for glomerulonephritis. Am J Kidney Dis. 2013;62:403–41.
65. Jones G, Juszczak M, Kingdon E, Harber M, Sweny P, Burns A. Treatment of idiopathic membranoproliferative glomerulonephritis with mycophenolate mofetil and steroids. Nephrol Dial Transplant. 2004;19:3160–4.
66. Yuan M, Zou J, Zhang X, Liu H, Teng J, Zhong Y, Ding X. Combination therapy with mycophenolate mofetil and prednisone in steroid-resistant idiopathic membranoproliferative glomerulonephritis. Clin Nephrol. 2010;73:354–9.
67. De S, Al-Nabhani D, Thorner P, Cattran D, Piscione TD, Licht C. Remission of resistant MPGN type I with mycophenolate mofetil and steroids. Pediatr Nephrol. 2009;24:597–600.
68. Bagheri N, Nemati E, Rahbar K, Nobakht A, Einollahi B, Taheri S. Cyclosporine in the treatment of membranoproliferative glomerulonephritis. Arch Iran Med. 2008;11:26–9.
69. Haddad M, Lau K, Butani L. Remission of membranoproliferative glomerulonephritis type I with the use of tacrolimus. Pediatr Nephrol. 2007;22:1787–91.
70. Kiyomasu T, Shibata M, Kurosu H, Shiraishi K, Hashimoto H, Hayashidera T, Akiyama Y, Takeda N. Cyclosporin A treatment for membranoproliferative glomerulonephritis type II. Nephron. 2002;91:509–11.
71. Daina E, Noris M, Remuzzi G. Eculizumab in a patient with dense-deposit disease. N Engl J Med. 2012;366:1161–3.
72. McCaughan JA, O'Rourke DM, Courtney AE. Recurrent dense deposit disease after renal transplantation: an emerging role for complementary therapies. Am J Transplant. 2012;12:1046–51.
73. Fakhouri F, de Jorge EG, Brune F, Azam P, Cook HT, Pickering MC. Treatment with human complement factor H rapidly reverses renal complement deposition in factor H-deficient mice. Kidney Int. 2010;78:279–86.
74. Paixao-Cavalcante D, Hanson S, Botto M, Cook HT, Pickering MC. Factor H facilitates the clearance of GBM bound iC3b by controlling C3 activation in fluid phase. Mol Immunol. 2009;46:1942–50.
75. Hegasy GA, Manuelian T, Hogasen K, Jansen JH, Zipfel PF. The molecular basis for hereditary porcine membranoproliferative glomerulonephritis type II: point mutations in the factor H coding sequence block protein secretion. Am J Pathol. 2002;161:2027–34.
76. Jansen JH, Hogasen K, Grondahl AM. Porcine membranoproliferative glomerulonephritis type II: an autosomal recessive deficiency of factor H. Vet Rec. 1995;137:240–4.
77. Hogasen K, Jansen JH, Mollnes TE, Hovdenes J, Harboe M. Hereditary porcine membranoproliferative glomerulonephritis type II is caused by factor H deficiency. J Clin Invest. 1995;95:1054–61.
78. Licht C, Heinen S, Jozsi M, Loschmann I, Saunders RE, Perkins SJ, Waldherr R, Skerka C, Kirschfink M, Hoppe B, Zipfel PF. Deletion of Lys224 in regulatory domain 4 of Factor H reveals a novel pathomechanism for dense deposit disease (MPGN II). Kidney Int. 2006;70:42–50.
79. Radhakrishnan S, Lunn A, Kirschfink M, Thorner P, Hebert D, Langlois V, Pluthero F, Licht C. Eculizumab and refractory membranoproliferative glomerulonephritis. N Engl J Med. 2012;366:1165–6.
80. Buttner-Mainik A, Parsons J, Jerome H, Hartmann A, Lamer S, Schaaf A, Schlosser A, Zipfel PF, Reski R, Decker EL. Production of biologically active recom-

binant human factor H in Physcomitrella. Plant Biotechnol J. 2011;9:373–83.
81. Schmidt CQ, Slingsby FC, Richards A, Barlow PN. Production of biologically active complement factor H in therapeutically useful quantities. Protein Expr Purif. 2011;76:254–63.
82. Martinez-Barricarte R, Heurich M, Valdes-Canedo F, Vazquez-Martul E, Torreira E, Montes T, Tortajada A, Pinto S, Lopez-Trascasa M, Morgan BP, Llorca O, Harris CL, Rodriguez de Cordoba S. Human C3 mutation reveals a mechanism of dense deposit disease pathogenesis and provides insights into complement activation and regulation. J Clin Invest. 2010;120:3702–12.
83. Licht C, Schlotzer-Schrehardt U, Kirschfink M, Zipfel PF, Hoppe B. MPGN II – genetically determined by defective complement regulation? Pediatr Nephrol. 2007;22:2–9.
84. Licht C, Weyersberg A, Heinen S, Stapenhorst L, Devenge J, Beck B, Waldherr R, Kirschfink M, Zipfel PF, Hoppe B. Successful plasma therapy for atypical hemolytic uremic syndrome caused by factor H deficiency owing to a novel mutation in the complement cofactor protein domain 15. Am J Kidney Dis. 2005; 45:415–21.
85. McGinley E, Watkins R, McLay A, Boulton-Jones JM. Plasma exchange in the treatment of mesangiocapillary glomerulonephritis. Nephron. 1985;40:385–90.
86. Oberkircher OR, Enama M, West JC, Campbell P, Moran J. Regression of recurrent membranoproliferative glomerulonephritis type II in a transplanted kidney after plasmapheresis therapy. Transplant Proc. 1988;20:418–23.
87. Rose KL, Paixao-Cavalcante D, Fish J, Manderson AP, Malik TH, Bygrave AE, Lin T, Sacks SH, Walport MJ, Cook HT, Botto M, Pickering MC. Factor I is required for the development of membranoproliferative glomerulonephritis in factor H-deficient mice. J Clin Invest. 2008;118:608–18.
88. Pickering MC, Warren J, Rose KL, Carlucci F, Wang Y, Walport MJ, Cook HT, Botto M. Prevention of C5 activation ameliorates spontaneous and experimental glomerulonephritis in factor H-deficient mice. Proc Natl Acad Sci U S A. 2006;103:9649–54.
89. Vivarelli M, Pasini A, Emma F. Eculizumab for the treatment of dense-deposit disease. N Engl J Med. 2012;366:1163–5.
90. Gurkan S, Fyfe B, Weiss L, Xiao X, Zhang Y, Smith RJ. Eculizumab and recurrent C3 glomerulonephritis. Pediatr Nephrol. 2013;28:1975–81.
91. Bomback AS, Smith RJ, Barile GR, Zhang Y, Heher EC, Herlitz L, Stokes MB, Markowitz GS, D'Agati VD, Canetta PA, Radhakrishnan J, Appel GB. Eculizumab for dense deposit disease and C3 glomerulonephritis. Clin J Am Soc Nephrol. 2012;7: 748–56.
92. Herlitz LC, Bomback AS, Markowitz GS, Stokes MB, Smith RN, Colvin RB, Appel GB, D'Agati VD. Pathology after eculizumab in dense deposit disease and C3 GN. J Am Soc Nephrol. 2012;23:1229–37.
93. Hong Y, Zhou W, Li K, Sacks SH. Triptolide is a potent suppressant of C3, CD40 and B7h expression in activated human proximal tubular epithelial cells. Kidney Int. 2002;62:1291–300.
94. Wang J, Tang Z, Luo C, Hu Y, Zeng C, Chen H, Liu Z. Clinical and pathological features of dense deposit disease in Chinese patients. Clin Nephrol. 2012;78: 207–15.
95. Glicklich D, Matas AJ, Sablay LB, Senitzer D, Tellis VA, Soberman R, Veith FJ. Recurrent membranoproliferative glomerulonephritis type 1 in successive renal transplants. Am J Nephrol. 1987;7:143–9.
96. Saxena R, Frankel WL, Sedmak DD, Falkenhain ME, Cosio FG. Recurrent type I membranoproliferative glomerulonephritis in a renal allograft: successful treatment with plasmapheresis. Am J Kidney Dis. 2000;35:749–52.
97. Tomlanovich S, Vincenti F, Amend W, Biava C, Melzer J, Feduska N, Salvatierra O. Is cyclosporine effective in preventing recurrence of immune-mediated glomerular disease after renal transplantation? Transplant Proc. 1988;20:285–8.
98. Lien YH, Scott K. Long-term cyclophosphamide treatment for recurrent type I membranoproliferative glomerulonephritis after transplantation. Am J Kidney Dis. 2000;35:539–43.
99. Muczynski KA. Plasmapheresis maintained renal function in an allograft with recurrent membranoproliferative glomerulonephritis type I. Am J Nephrol. 1995;15:446–9.