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C3 glomerulopathy: Understanding an ultra-rare complement-mediated renal disease

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Abstract

C3 glomerulopathy (C3G) describes a pathologic pattern of injury diagnosed by renal biopsy. It is characterized by the dominant deposition of the third component of complement (C3) in the renal glomerulus as resolved by immunofluorescence microscopy. The underlying pathophysiology is driven by dysregulation of the alternative pathway of complement in the fluid-phase and in the glomerular microenvironment. Characterization of clinical features and a targeted evaluation for indices and drivers of complement dysregulation are necessary for optimal patient care. Autoantibodies to the C3 and C5 convertases of complement are the most commonly detected drivers of complement dysregulation, although genetic mutations in complement genes can also be found. Approximately half of patients progress to end-stage renal disease within 10 years of diagnosis, and, while transplantation is a viable option, there is high risk for disease recurrence and allograft failure. This poor outcome reflects the lack of disease-specific therapy for C3G, relegating patients to symptomatic treatment to minimize proteinuria and suppress renal inflammation. Fortunately, the future is bright as several anti-complement drugs are currently in clinical trials.

KEYWORDS

C3 glomerulonephritis, C3 glomerulopathy, dense deposit disease

1 | INTRODUCTION

C3 glomerulopathy (C3G) describes a pathologic pattern of injury diagnosed by renal biopsy. It is characterized by the dominant deposition of the third component of complement (C3) in the renal glomerulus as resolved by immunofluorescence (IF) microscopy. This finding, in the absence or near-absence of other immune-reactants in a patient with the classical clinical features of a glomerulonephritis, is required to make the diagnosis. The term C3G, itself, was assigned by a panel of experts in 2013, although the pattern of injury has been recognized for decades (Banks, May, & Willington, 1982; Galle & Mahieu, 1975; Habib, Gubler, Loirat, Ma1z, & Levy, 1975; Kim, Vernier, Fish, & Michael, 1979). Adding an electron microscopic evaluation to IF facilitates the recognition of two subgroups of C3G, dense deposit disease (DDD), and C3 glomerulonephritis (C3GN). Each subgroup is defined by the specific localization pattern and characteristics of deposits within renal tissue.

Glomerular deposition of C3 reflects activation of the alternative pathway (AP) of complement, and consistent with this underlying pathophysiology. In C3G patients, AP dysregulation can be documented in the circulation (so called fluid-phase dysregulation) and/or in the glomerular microenvironment. Fluid-phase dysregulation is presumed to lead to complement dysregulation in the glomerular

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microenvironment. This article presents our current understanding of C3G. After reviewing the complement system, we focus on the clinical presentation of C3G, discuss drivers of disease, and conclude with an overview of therapeutic options.

2 | THE COMPLEMENT CASCADE

The complement system is the cornerstone of innate immunity. It is the first line of defense against foreign and altered host cells, leads to activation of adaptive immunity, and provides integrated crosstalk with multiple other pathways, including the coagulation pathway. Activation is facilitated by three different initiating pathways: the classical pathway (CP), lectin pathway (LP), and AP. An amplification phase follows the initiation phase and segues into the terminal or effector phase of complement (Figure 1). In aggregate, over 30 individual proteins work in concert to defend against microorganisms, eliminate immune complexes and apoptotic cells from the circulation, and modulate adaptive immune responses (Carroll, 2008).

Initiation of complement activity is associated with pathway-specific triggers. For example, activation of the CP involves recognition of target clusters such as pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), and antibody complexes, while the LP is activated through recognition of microbial polysaccharides. Upon recognition of these triggers, C1r/C1s proteases and MASP1-3 (mannose binding ligand-associated serine proteases), respectively, are activated to cleave C4 to C4a and C4b and C2 to C2a and C2b. The consequence is the formation of C4b2a, the C3 convertase of the CP and LP (Ricklin, Reis, Mastellos, Gros, & Lambris, 2016).

In comparison, the AP is constitutively active through the spontaneous hydrolysis of a thioester bond on C3 to produce C3(H_2O). This process is termed tick-over and occurs at a rate of ~1% of total C3 per hour (Smith, Harris, & Pickering, 2011; Zipfel et al., 2015). C3(H2O) is conformationally altered enabling it to interact with and bind to factor B (FB) to form C3(H2O)B, which is cleaved by factor D (FD) to generate C3(H_2O)Bb, also a C3 convertase. At this point, all three pathways converge to generate C3bBb, the C3 convertase of the AP (Figure 1). While all C3 convertases cleave C3 to C3a and C3b, the AP C3 convertase, C3bBb, is the major driver of the complement response and accounts for ~80% of complement activity (Harboe, Ulvund, Vien, Fung, & Mollnes, 2004). As copious amounts of C3 are cleaved, the local density of C3b increases and with binding of C3b to C3bBb, C3bBbC3b forms. This complex, a C5 convertase, cleaves C5–C5a, which is a potent anaphylatoxin, and C5b, which associates with C6–C8 and several C9 units to form a lytic complex termed membrane attack complex (MAC) and/or soluble C5b–C9 (sC5b–C9) (Nester & Smith, 2016; Zipfel, Zipfel, & Strauß, 2020; Zipfel et al., 2015).

Complement activity is tightly controlled by numerous proteins known collectively as regulators of complement activity (RCAs), which are present in the fluid phase or are membrane bound. Fluid phase regulators include C4 binding protein (C4BP), vitronectin (S protein), clusterin, factor I (FI), factor H (FH), and factor H-like 1 (FHL-1; Noris & Remuzzi, 2015; Turkmen, Baloglu, & Ozer, 2021), while decay accelerating factor (DAF or CD55), membrane cofactor protein (MCP or CD46), complement receptor 1 (CR1 or CD35), and CD59 (Carroll, 2008; Ricklin et al., 2016; Turkmen et al., 2021) are examples of membrane bound RCAs (Figure 2).

The RCA that is most relevant to C3G is FH, a linear protein comprised of 20 short consensus repeat (SCR) units each about 60 amino acids in length connected one to another by short linker regions (Figure 3). The four amino-terminal SCRs of FH can bind to C3b, enabling FH to regulate complement activity at the level of C3 convertase by three mechanisms: (a) cleavage of C3b to inactive C3b (iC3b) through cofactor activity with FI, (b) decay accelerating activity (DAA) by sterically displacing Bb from the C3 convertase (C3bBb), and (c) inhibition of C3 convertase formation by competing with FB for binding with C3b (Reid et al., 1986). The two carboxy-terminal SCRs of FH, SCRs 19–20, recognize, and bind heparan sulfate (HS) on the glomerular basement membrane (GBM; Figure 4). Factor H like-1 (FHL-1) is a short isoform of FH that includes the first seven SCRs and then a unique four amino acid C-terminus (Figure 3; Cserhalmi, Papp, Brandus, Uzonyi, & Józsi, 2019). Although the role of FHL-1 in the glomerular microenvironment is unclear, in the eye its small size allows it to diffuse passively through Bruch's membrane while the much larger, glycosylated, FH cannot (Clark et al., 2014).

In addition to FH, there are five factor H-related proteins (FHRs) the functions of which are largely unknown, making their role in different complement-mediated diseases unclear (Poppelaars et al., 2021). They are divided into type 1 FHRs (FHR1, FHR2, and FHR5), which occur as homo- and heterodimers, and type 2 FHRs (FHR3, FHR4A, and FHR4B), which are monomeric in plasma (Figure 3; Goicoechea De Jorge et al., 2013; Lorés-Motta et al., 2021). Type 1 FHRs, in particular FHR1 and FHR5, are prominently found in renal biopsies of C3G patients (Sethi et al., 2009). In addition, pathologic type 1 FHR gene rearrangements that generate novel FHRs proteins are implicated in genetically driven types of C3G (Xiao, Pickering, & Smith, 2014).

In toto, the fine balance between activators and regulators of complement prevents uncontrolled complement-mediated damage to oneself while robustly defending against microorganisms, eliminating immune complexes and apoptotic cells from the circulation, and modulating the adaptive immune response.

3 | DISEASE PRESENTATION AND DIAGNOSIS

3.1 | Disease presentation

C3G presents as a glomerulonephritis (GN), with the classical constellation of signs and symptoms, including hematuria, proteinuria, edema, and often hypertension; in most patients, serum C3 levels are also low. The differential diagnosis for GN is broad and if symptoms persist and C3 levels remain low for more than 3 months, a renal biopsy is warranted. If the renal biopsy shows C3 dominant

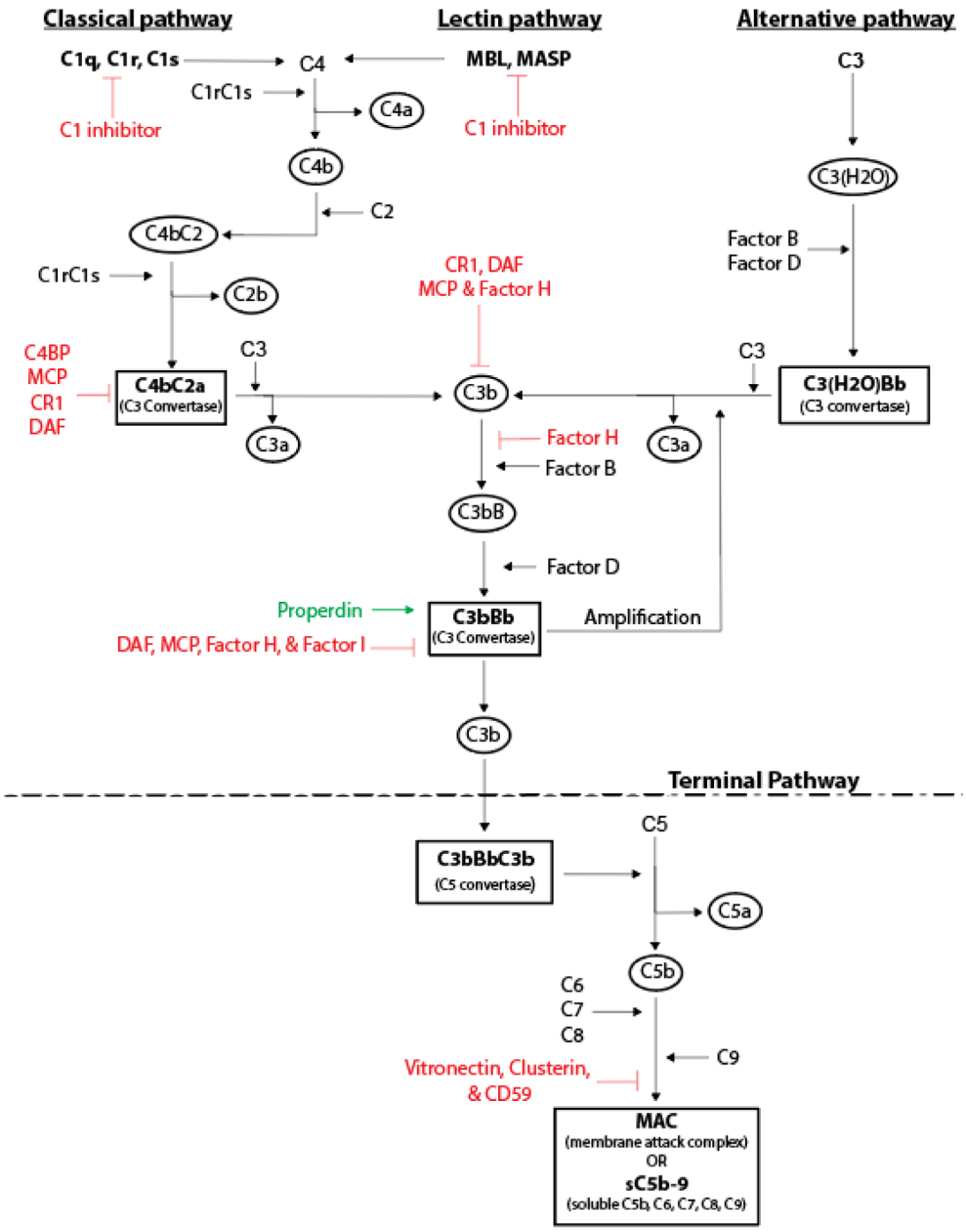

FIGURE 1 The complement cascade in C3 glomerulopathy. The complement pathway is activated through three pathways—the classical (CP), lectin (LP), and alternative (AP). The CP is induced by the complement component 1 (C1) complex binding to antibody complexes, pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs), whereas the LP recognizes microbial polysaccharides. Upon activation, complement component 4 (C4) and complement component 2 (C2) are cleaved by proteases to form C4bC2a, the C3 convertase of the CP and LP. The AP is activated by the spontaneous hydrolysis of complement component 3 (C3) to form C3(H_2O), which interacts with factor B and factor D to form C3(H2O)Bb, the initial C3 convertase of the AP. C3 convertases of the CP, LP, and AP cleave C3 into C3a and C3b. C3b binds with factor B and is cleaved by factor D to form C3bBb, the major C3 convertase of the AP. C3bBb cleaves additional C3–C3b and C3a amplifying the complement response. As local levels of C3b increase, C3b associates with C3bBb to form C3bBbC3b, the C5 convertase of the AP. C5 convertase cleaves C5–C5a and C5b. C5b associates with complement components 6–8 (C6–8) and complement component 9 (C9) to from membrane attack complex, which lyses cells, or soluble C5b–9, which is cleared from circulation. C3 glomerulopathy is caused by dysregulation of the AP, although a small percentage of cases are triggered by the CP and LP. Regulators of complement are shown in red, and the sole activator of the complement system, properdin, is shown in green

deposition (at least 2 orders of magnitude greater than any other immunoreactant) in the renal glomerulus as resolved by IF, C3 dominant glomerulopathy (C3 DG) can be diagnosed. Included under the diagnosis of C3DG is post-infectious glomerulonephritis (PIGN), monoclonal gammopathy of renal significance (MGRS), and C3G. Electron microscopy (EM) facilitates the recognition of two subgroups of C3G, DDD and C3GN, each defined by the specific localization pattern and characteristics of deposits within renal

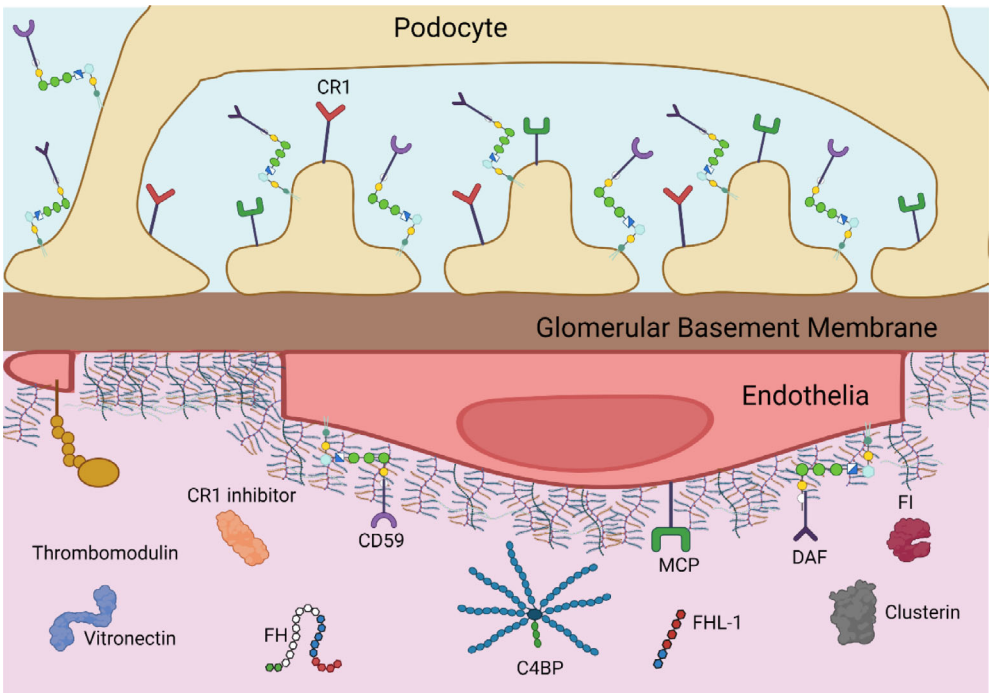

FIGURE 2 Regulators of complement in the glomerular microenvironment. Fluid phase and membrane-bound regulators play a pivotal role in protection and selectivity of complement activity in the glomerular microenvironment. CR1 inhibitor and C4BP are fluid phase regulators of the CP and LP. Fluid phase regulators of the AP include FH, FHL-1, and FI. The functions of FH include destabilization of C3bBb by decay accelerating activity, inhibition of FB binding to C3b, and cofactor activity with FI to inactivate C3b to iC3b. The FH splice variant, FHL-1, also has decay accelerating activity and cofactor activity. DAF, MCP, and CR1 are membrane-bound regulators of the AP that destabilize C3bBb through cofactor activity and/or decay accelerating activity. Additionally, thrombomodulin is a membrane-bound regulator that uniquely enhances FH cofactor activity for C3 convertase dissociation. CD59, vitronectin (S protein) and clusterin inhibit the terminal pathway by preventing MAC formation. Thrombomodulin, MCP and CR1 are transmembrane receptors while CD59 and DAF are GPI-anchored receptors. AP, alternative pathway; C1, complement component 1; C3, complement component 3; C4BP, complement component 4 binding protein; CP, classical pathway; CR1, Complement receptor 1; DAF, decay accelerating factor; FB, factor B; FH, factor H; FHL-1, factor H-like 1; FI, factor I; GPI, glycosylphosphatidylinositol; LP, lectin pathway; MAC, membrane attack complex; MASP1-2, mannose binding ligand associated serine proteases 1–2; MCP, membrane cofactor protein

tissue (Figure 5). It is important to recognize that biopsies can change from immunocomplex membranoproliferative glomerulonephritis (IC-MPGN) to C3G, implying that the diagnosis of IC-MPGN does not rule out genetic or acquired drivers of AP dysregulation (Iatropoulos et al., 2018; Noris, Daina, & Remuzzi, 2021; Piras et al., 2021).

Average age at diagnosis of C3G is 21 years, however stratifying by subgroups, age at diagnosis is higher in C3GN than in DDD (about 30 and 19 years old, respectively) (Servais et al., 2012). Approximately ~60% of DDD and ~40% of C3GN patients have hypocomplementemia, with abnormalities of complement proteins and their cleavage products indicative of ongoing AP complement activity (Figure 6) (Nester & Smith, 2013; Smith et al., 2019). C3G is also associated with the development of drusen, making an ophthalmologic evaluation an essential part of an C3G patient's regular care (Nasr et al., 2009). While the natural history of C3G is not well defined, hematuria, proteinuria, and nephrotic syndrome are the most common signs and symptoms (Caravaca-Fontán et al., 2021; Michels et al., 2022; Nakagawa et al., 2021; Pınarbaşı et al., 2021). About ~75% of pediatric C3G patients present with low C3 as compared to ~50% of adults. Estimated glomerular filtration rate is also significantly lower in adults, perhaps reflecting the increased glomerulosclerosis observed on biopsy (Zahir, Wani, Gupta, & Agrawal, 2020). About one-third of adults present with acute kidney failure, a presentation rarely seen in pediatric C3G patients (Zahir et al., 2020). These findings suggest that C3G may be a more aggressive disease in the adult population, however this hypothesis, while supported by some studies, is refuted by others (Lu, Moon, Lanning, McCarthy, & Smith, 2012; Nasr et al., 2009). In aggregate, ~30–50% of C3G patients progress to ESRD within 10 years of diagnosis. If transplantation is offered, histologic disease recurrence is the rule (~90%) with allograft loss occurring in about half of the cases (Servais et al., 2012).

3.2 | Clinical evaluation

A recent publication by the KDIGO Glomerular Diseases Work Group describes the practice strategy relevant to the evaluation and

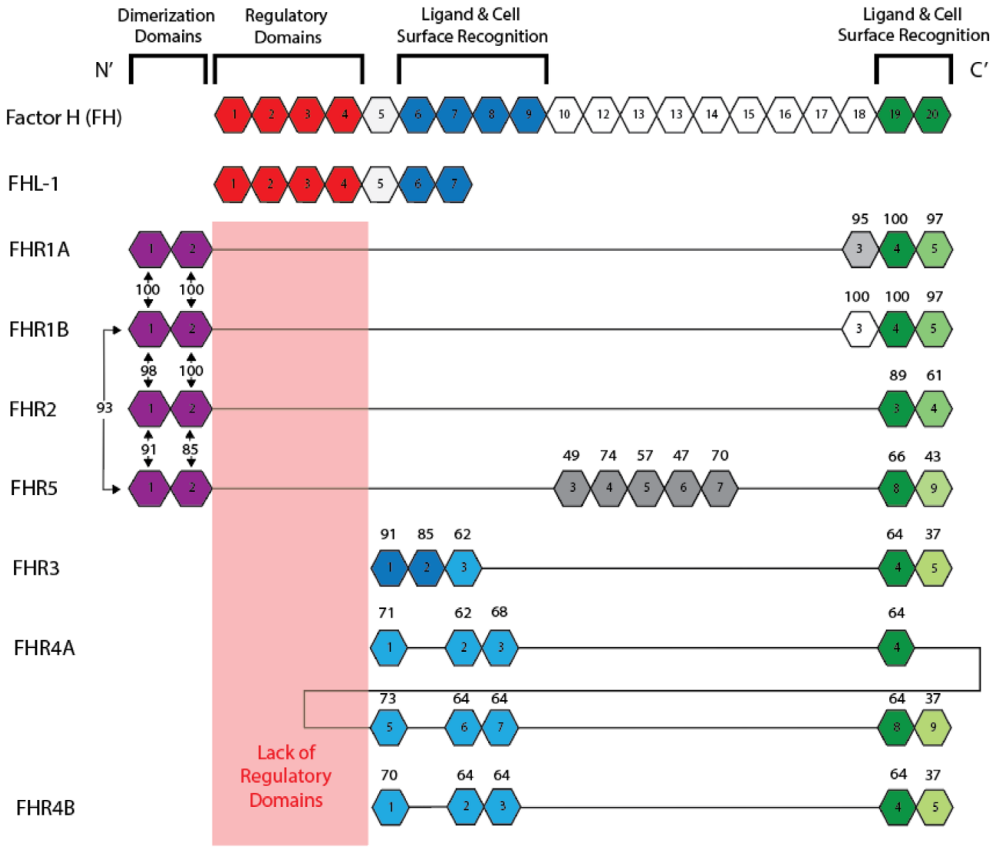

FIGURE 3 The Factor H protein family in humans. All members of the factor H (FH) protein family are built on modules of ~60 amino acids called SCRs that have specific functions. FH has 20 SCRs. The four N′-terminal SCRs (SCRs 1–4) mediate complement regulation, while SCRs 6–9 and the C′-terminal SCRs 19–20 recognize ligands in the glycocalyx such as heparan sulfate (HS). Factor H like 1 (FHL-1) is a splice variant of FH that shares N′-terminal regulatory functions with FH. SCRs 6–7 of FHL-1 also recognize ligand and cell surfaces like FH SCRs 6–8. In comparison, factor H-related proteins (FHRs) 1, 2, and 5 (called type 1 FHRs) lack the N′-terminal regulatory domains of FH but do have similar C′-terminal SCRs. Like SCRs 19–20 of FH, the last two domains of type 1 FHRs recognize cell surface ligands such as HS. The N′-terminal SCRs 1–2 of the type 1 FHRs are essential for homo- and hetero-dimerization. Type 2 FHRs (FHR3 and FHR4) lack regulatory domains but share sequence similarity with the last two SCRs of FH. As a consequence of this sequence similarity, FH and FHRs functionally compete in the local glomerular microenvironment. (In this figure, SCRs are represented by hexagons and aligned by sequence similarity. Amino acid similarities are indicated by number percentages relative to FH or to dimerization domains of type 1 FHRs. Binding and activation sites are indicated by color. A and B for FHR1 indicate acidic and basic forms. A and B for FHR4 indicate long and short isoforms)

treatment of patients with C3G (Rovin et al., 2021). A major focus should be on identifying the underlying cause of disease and therefore PIGN and, in patients over the age of 50, MGRS should be excluded before assigning the diagnosis of C3G. If no underlying etiology for disease is found after an extensive work-up, a thorough evaluation for both complement dysregulation and the drivers of this dysregulation should be completed.

The evaluation for complement dysregulation should include serum levels of complement proteins and their cleavage products as well as functional assays of AP and CP integrity and activity. To identify possible drivers of this dysregulation, patients should be screened for nephritic factors (C3, C4, and C5), autoantibodies to FH and FB, and genetic variants in *C3*, *CFH*, *CFI*, *CFB*, *DGKE*, and *CFHR1-5* that can lead to dysregulation of the AP (Table 1). By determining the degree and the cause of AP dysregulation, treatment plans can be tailored to provide patients with the best possible renal outcome.

3.3 | Drivers of disease

3.3.1 | Acquired drivers

The majority of C3G patients are positive for autoantibodies against complement convertases or specific complement proteins that impair normal convertase or protein function. Testing for these autoantibodies is available only through select laboratories as the assays to identify and quantify activity are highly specialized. If identified, serial testing of autoantibodies is recommended as activity and titer change over time, which may be relevant to long-term outcome.

Nephritic factors are autoantibodies that recognize neoepitopes on C3 and C5 convertases. C3 nephritic factors (C3Nefs) against C3bBb are most common, and are reported in 50–80% of DDD patients and 44–50% of C3GN patients (Iatropoulos et al., 2016; Servais et al., 2012; Yuzhou Zhang et al., 2012). By protecting C3bBb from FH-mediated decay, C3Nefs stabilize and prolong the half-life of

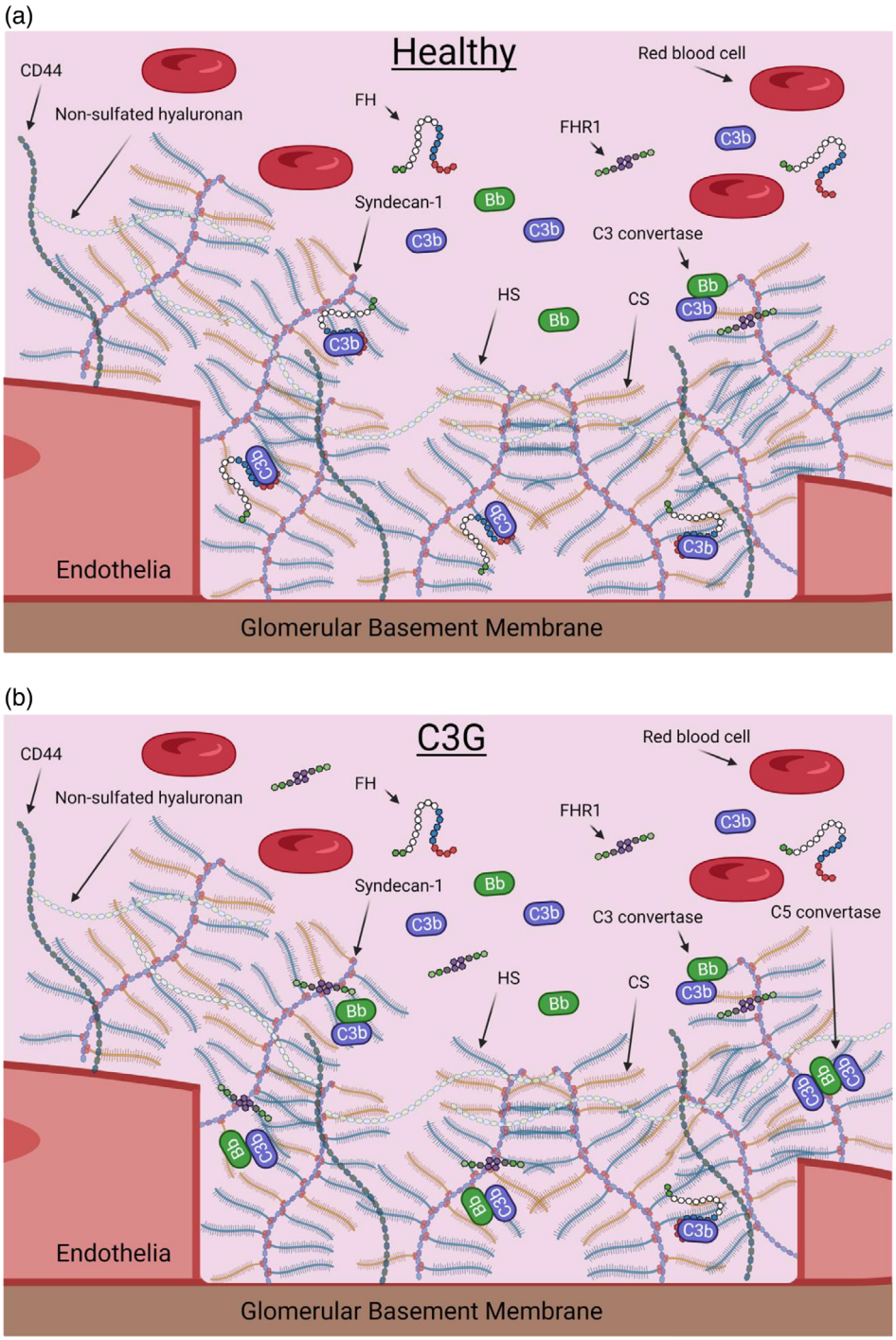

FIGURE 4 Complement in the glycocalyx. Complement effects on C3 glomerulopathy (C3G) are most prominent in the glomerular microenvironment. Within a kidney nephron, the glomerulus is the filtration unit and is made up of several filtration layers, including the glycocalyx, endothelial cells, glomerular basement membrane (GBM), and podocytes. Together, they retain valuable proteins and filter solutes/waste products into the Bowman's space to be excreted as urine. The glycocalyx, the first filtration barrier, is a highly charged layer of membrane-bound macromolecules attached to glomerular endothelial cell and the GBM overlying the glomerular endothelial pores, which are termed fenestrae. The glycocalyx is composed of glycosaminoglycans and proteoglycans linked to the cytoskeleton to provide stability as well as cell signaling. Important glycosaminoglycans to note are heparan sulfate (HS), chondroitin sulfate (CS), and nonsulfated hyaluronan (Martinez-Seara Monne, Danne, Róg, Ilpo, & Gurtovenko, 2013). Nonsulfated hyaluronan forms a stiff, random coil that allows small molecules to diffuse freely while excluding larger molecules. HS and CS are distributed on all cell surfaces and in extracellular matrices and bind a variety of ligands (Izumikawa & Kitagawa, 2010). HS in particular binds factor H (FH) and factor H-related proteins (FHRs; Blackmore et al., 1998; Clark et al., 2014; Skerka, Chen, Fremeaux-Bacchi, & Roumenina, 2013). FH binding regulates formation of C3 convertase while FHRs promote C3 convertase activation. The local balance between FH and FHRs impacts complement control and when altered can potentiate glomerular disease (Csincsi et al., 2017; Goodship et al., 2017; Loeven et al., 2021; Tortajada et al., 2013). (a) The balance between FH and FHRs favors complement control. (b) Increased binding of FHRs (FHR1 is shown) and decreased binding of FH drives complement dysregulation

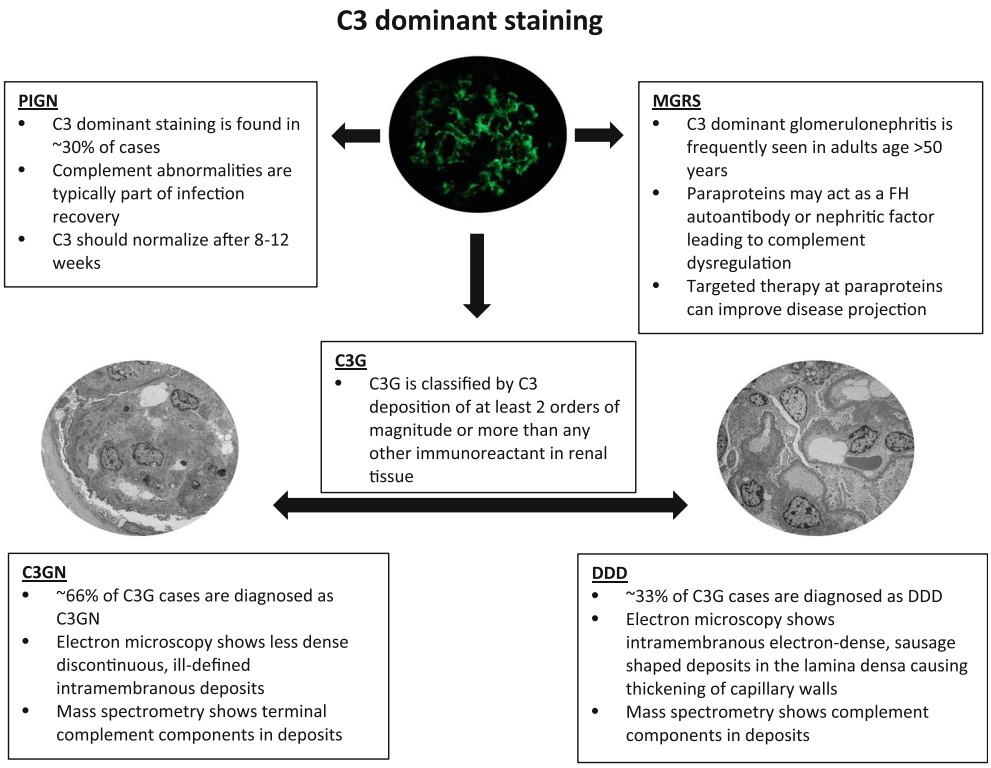

FIGURE 5 Differential diagnosis of C3 dominant glomerulonephritis. Complement component 3 (C3) dominant staining is defined as C3 staining greater by at least two orders of magnitude more than any other immunoreactant in renal tissue. Disease processes that fall into this category are monoclonal gammopathy of renal significance (MGRS), post-infectious glomerulonephritis (PIGN), and C3 glomerulopathy (C3G). If C3 dominant glomerulonephritis is diagnosed, patients >50 years of age should be screed for paraproteins. During screening, protease digestion prior to IF can unmask immunoglobulins (Igs). This treatment will help avoid misdiagnosis of C3G, as shown by recent studies demonstrating false-negative staining of Igs on routine IF (Larsen et al., 2015). Ig staining equal to C3 staining at first biopsy; however, does not rule out genetic or acquired drivers of AP dysregulation. C3 dominant staining is also observed in ∼30% of PIGN, which resembles C3G on renal biopsy making it necessary to monitor C3 serum levels for normalization. If C3 levels normalize after ∼8 weeks PIGN is the likely diagnosis; if C3 levels remains abnormal for 12 weeks or greater, a biopsy is warranted and high on the differential diagnosis is C3G. C3G is a pathological description of a disease process that includes two subgroups defined by differences on electron microscopy (EM)–C3 glomerulonephritis (C3GN) and dense deposits disease (DDD). C3GN accounts for ∼66% of C3G cases and on EM has mesangial, subepithelial, subendothelial, and/or less dense, discontinuous, ill-defined intramembranous deposits. DDD accounts for ∼33% of C3G cases and on EM has findings of sausage-shaped, electron dense intramembranous deposits that thicken the lamina densa. Light microscopy cannot differentiate C3G from other glomerulonephritis

this convertase in the fluid phase, typically leading to an associated reduction in serum levels of C3 with a concomitant increase in levels of its cleavage product, C3c (Servais et al., 2012; Xiao et al., 2014).

C5 nephritic factors (C5Nefs) are also common. These autoantibodies bind to and stabilize C3bBbC3b, increasing the half-life of the C5 convertase. This increase, in turn, leads to C5 consumption and frequently the generation of sC5b–9. The likelihood of co-positivity for both C3Nefs and C5Nefs is high. As a general rule, C5Nefs are more frequently associated with C3GN than DDD (Marinozzi et al., 2017).

Less frequently, C4 nephritic factors (C4Nefs), which bind to and stabilize C4b2a, are identified (Halbwachs, Leveilla, Lesavre, Wattel, & Leibowitch, 1980; McLean & Nilson, 1979). In addition to nephritic factors, autoantibodies against FH and FB are occasionally detected (Chiara Marinozzi et al., 2017). These autoantibodies impair FH regulation and stabilize the C3 convertase against FH mediated decay, respectively (Blanc et al., 2015; Chen, Muller, Rudolph, et al., 2011; Goodship et al., 2012; Strobel, Zimmering, Papp, Prechl, & Józsi, 2010).

3.4 | Genetic drivers

Approximately 25% of C3G patients carry rare variants or genomic rearrangements in complement genes that are disease-associated, making genetic testing an important part of the diagnostic algorithm (Servais et al., 2012). Testing should focus on genes implicated in C3G, which include *C3*, *CFB*, *CFH*, *CFI*, *DGKE*, and *CFHR5*. In addition, the *CFHR* genomic region should be screened for complex rearrangements typically by multiplex-ligation-dependent probe amplification (MLPA), a multiplex PCR-based method that can detect the copy number of each *CFHR* gene. When exon-specific probes are used, *CFHR* genomic rearrangements such as the *CFHR5* fusion gene endemic to Cyprus can be easily identified (Gale et al., 2010; Garam et al., 2021; Schouten et al., 2002).

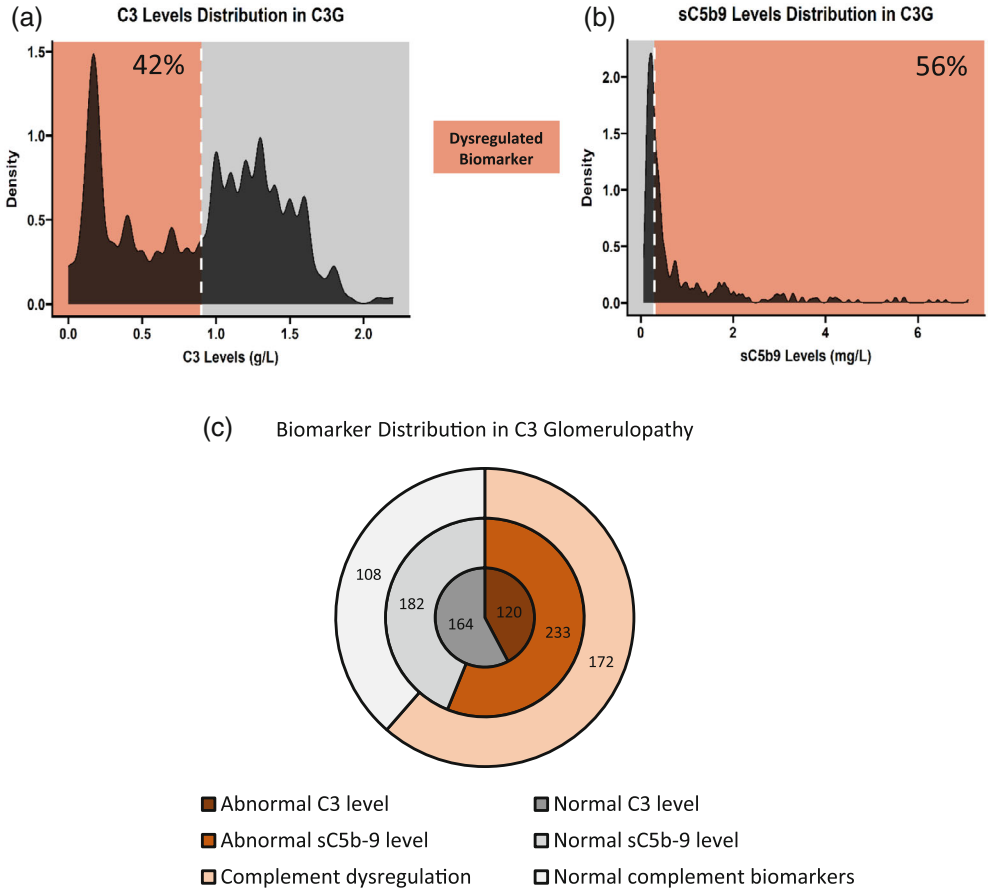

FIGURE 6 Biomarker distribution of C3 and soluble C5b-9 in C3 glomerulopathy. (a) Of 285 C3 glomerulopathy (C3G) patients, 42% had low complement component 3 (C3) serum levels providing fluid-phase evidence of increased complement activity at the level of the C3 convertase, however in 58% serum C3 was normal. (b) Of 416 C3G patients, 56% had increased soluble C5b-9 (sC5b-9) levels, a fluid-phase indication of terminal pathway activity; in 44%, sC5b-9 was normal. (c) In aggregate, of 280 patients with both sC5b-9 and C3 testing, 172 (61.4%) had fluid-phase evidence of complement dysregulation, while in 108 patients (38.6%), circulating levels of complement components were normal

TABLE 1 Comprehensive evaluation of the alternative pathway

Biomarkers	C3, C3b, C3c, C4, C5, FI, FH, FB, bb, and properdin
Acquired drivers	C3Nefs, C4Nefs, C5Nefs, FHAA, and FBAA
Functional assays	CH50, APFA, and FH function
Genetic testing	*CFH*, *CFI*, *CFB*, *CFHR5*, *C3*, *DGKE*, and *CFHR1-CFHR5* MLPA

Note: A comprehensive evaluation of complement parameters is recommended in all C3 glomerulopathy patients. Testing is complex and often requires specialized laboratories to run the assays and consultation with experts for interpretation of results. A genetic renal panel is recommended to identify mutations that may drive complement dysregulation.
Abbreviations: APFA, alternative pathway functional assay; Bb, cleavage product of factor B; C3, Complement component 3; C3Nefs, complement component 3 nephritic factor; C4, complement component 4; C4Nefs, complement component 4 nephritic factors; C5, complement component 5; C5Nefs, complement component 5 nephritic factors; *CFHR1–CFHR5*, complement factor H-related 1–5; *CFHR5*, complement factor H-related 5; CH50, classical pathway function assay; *DGKE*, diacylglycerol kinase epsilon; FB, factor B; FBAA, factor B autoantibodies; FH, factor H; FHAA, factor H autoantibodies; FI, factor I; MLPA, multiplex-ligation-dependent probe amplification.

It is important to recognize that while few families segregate C3G as a Mendelian disease, this scarcity does not mean that genetic contributions to C3G should be minimized or overlooked. Rather, the dearth of families is reflective of variability in variant penetrance secondary to both the complexity of the complement system and the impact differing serum levels of complement proteins have on the disease phenotype. By way of example, the ultrarare variant in *CFB*, c.1101C>G, p.Ser367Arg, is a gain-of-function mutation that impairs FH-mediated regulation of C3bBb. C3bBb carrying mutant FB p.R367 requires higher concentrations of FH for effective DAA. The variant has been reported in a Japanese family in which a mother and one child have C3G and in two unrelated Caucasian patients who developed atypical hemolytic uremic syndrome (aHUS), one as an infant and the other in adulthood. With respect to the aHUS phenotype, a germane finding that explains the variation in time-of-disease onset in these two patients is their FH serum levels: the child who developed disease at 10 months of age had borderline low FH levels, while in the adult patient, FH levels were twice as high, a difference that is explained at least in part by the *CFH*-H3 risk haplotype found in the child (Zhang et al., 2020). This haplotype is associated with lower FH levels and predisposes to complement dysregulation. The consequence is that DAA is more profoundly compromised in the child, leading to early onset disease. With respect to the Japanese family, the persons with C3G also carried two other rare genetic variants in complement genes—one in *C3* (c.2746G>A, p.V916I; minor allele frequency 0.000054) and the other in *CFI* (c.603A>C, p.R201S; minor allele frequency 0.0011), suggesting that these additional variants

drive the phenotype to C3G (Imamura et al., 2015; Martínez-Barricarte et al., 2010).

In addition to screening for single nucleotide variations and small insertions and deletions in complement genes, genetic testing should include an evaluation of *CFHR* genes for complex rearrangements. These changes often generate novel fusion proteins that cause C3G by a gain-of-function mechanism that drives AP dysregulation in the glomerular microenvironment. The tick-over process mentioned earlier leads to a low level of normal C3 activation within glomeruli. The glomerular endothelial cells have membrane-bound RCA proteins to control tick-over; however, the glomerular endothelial cell fenestrations do not. These transcytoplasmic holes depend on circulating RCA proteins, and in particular, FH to control the tick-over process (Satchell & Braet, 2009). If FH function is impaired or the balance between FH and FHRs is altered, the glycocalyx overlying the fenestrae becomes a complement-activating surface and abnormal C3 accumulation and AP activity occur.

The prototypical example of this process is CFHR5 nephropathy, a subtype of C3GN that is endemic to Cyprus, where it affects ~1 in 6,000 persons. Affected persons carry one copy of a *CFHR5* gene in which there is an internal duplication of the exons encoding the first two SCRs of the FHR5 protein. The result is a novel FHR5 protein that has 11 SCRs instead of the usual nine (Malik et al., 2021). The mutant protein shows increased avidity for C3 ligands and interacts with surface C3 within glomeruli more efficiently than FH, thereby promoting complement activation through a gain-of-function mechanism. The clinical hallmark of this process is microscopic hematuria, almost always exacerbated by respiratory infections. About 25–50% of affected persons report macroscopic hematuria as well. Importantly, there is generally no evidence of systemic complement dysregulation; rather, the AP dysregulation is confined to the glomerular microenvironment. The eventual outcome is that over 80% of males but only a small proportion of females suffer a stepwise deterioration in renal function that leads to ESRD usually between 30 and 70 years of age (Gale & Pickering, 2011). Why there is a male bias is currently not known.

The complexity of the genetics underlying C3G mandates testing in laboratories with the necessary expertise to provide a robust and vigorous interpretation of the genetic findings in each patient in the context of their clinical course and a systemic evaluation of complement activity.

4 | PATIENT EVALUATION AND TREATMENT

An optimal treatment for C3G patients has not been established; however, there are guideline recommendations to support renal health, most recently those published by the KDIGO Glomerular Diseases Work Group, which reflect expert opinion and the collective experience derived from a number of clinical case series (Rovin et al., 2021). The overarching premise is that treatment depends on identifying the underlying cause of disease by following a focused but thorough investigation. Since C3G is only a pathologic description of a pattern of injury seen on renal biopsy that is characterized by the dominant deposition of C3 in the renal glomerulus, the first steps toward a C3G diagnosis are to rule out PIGN and in persons over 50 years of age, MGRS. If no cause of disease is found, patients should be evaluated for both complement dysregulation and drivers of complement dysregulation, with subsequent care based on clinical presentation (Figure 7).

4.1 | Nonspecific treatments

Angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin receptor blockers (ARBs) are first line treatments for proteinuria and blood pressure control as their use has been shown to improve renal survival (Servais et al., 2012; Turkmen et al., 2021). Lipid-lowering agents can also be considered. If proteinuria increases, general immunosuppressive therapy with mycophenolate mofetil (MMF) plus steroids should be considered.

The theoretical benefit offered by MMF derives from its inhibition of T-cell and B-cell proliferation, which in turn should blunt cell-mediated immune responses and autoantibody formation (Allison, 2005; Ravindran, Fervenza, Smith, De Vriese, & Sethi, 2018). The reported effectiveness of this treatment, however, is mixed. Studies by Caravaca-Fontain et al. and Rabasco et al. noted higher rates of remission and a lower probability of kidney failure in C3G patients on MMF and corticosteroids as compared to other immunosuppressive regimens (Caravaca-Fontán et al., 2020; Rabasco et al., 2015). Avasare et al. and Ravindran et al., however, reported more varied outcomes with MMF-based regimens, including patients who fail to respond to this type of treatment (Avasare et al., 2018; Ravindran et al., 2018). In a comparative study of C3G patients on MMF-based treatment verse other immunosuppressive regimens, Caliskan et al. found no difference between treatment groups with respect to rate of kidney failure or decline in glomerular filtration rate from baseline (Caliskan et al., 2017).

In aggregate, these results suggest that while MMF plus steroids may be effective in some C3G patients; however, there is a pressing need for disease-specific treatments. For this reason, based on the pivotal role of complement dysregulation in driving the disease process, several pharmaceutical companies and academic research laboratories have focused on complement-based therapies as a treatment for C3G.

4.2 | Anti-complement therapies

The complexity of the complement system offers several therapeutic targets by which to modulate complement activity thereby preventing on-going dysregulation and altering the clinical course of C3G. Among the prime targets are C3, FB, and FD, and clinical trials testing inhibitors against each of these proteins are on-going (see ClinicalTrials.gov). Theoretically, targeting C3, FB, and FD should prevent formation of the C3 convertase, C3bBb. Preventing C3 convertase formation

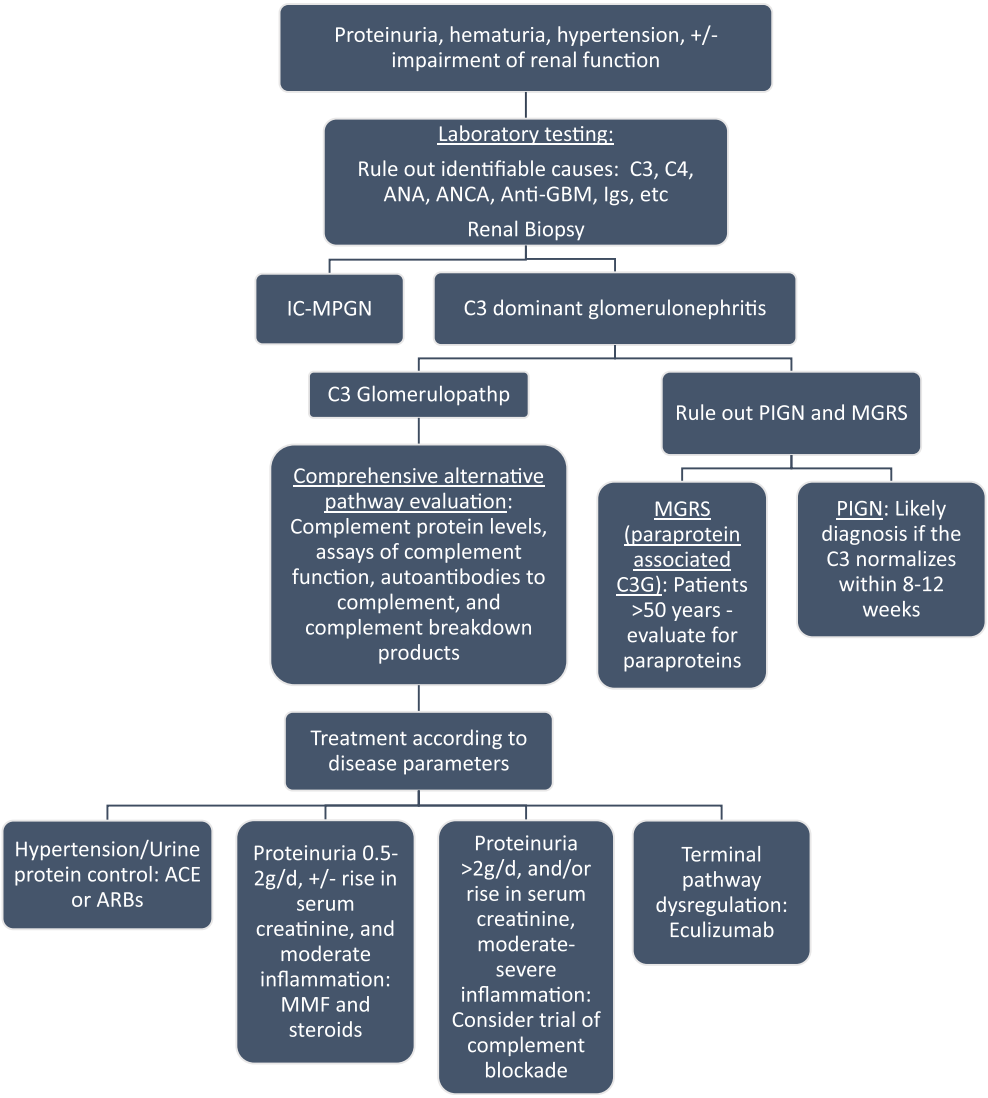

FIGURE 7 Treatment algorithm for C3 glomerulopathy. Patients who present with proteinuria, hematuria (macroscopic or microscopic), hypertension, or signs and symptoms of renal function impairment (renal insufficiency, nephrotic syndrome, and/or nephritic syndrome) should have a complete blood count, complement component 3 (C3), complement component 4 (C4), antinuclear antibodies, antineutrophil cytoplasmic antibodies, and urine protein creatinine, as well as screening for hepatitis and other infections if indicated. Persistent proteinuria, a reduce glomerular filtration rate, and/or unexplained hypertension are considerations for kidney biopsy. Based on biopsy findings, current classification separates patients into IC-MPGN with significant glomerular Ig and complement deposition, and C3G with characteristic C3 dominant glomerulonephritis; however, recent evidence suggests some overlap between C3G and IC-MPGN. In patients with biopsy confirmed C3 dominant glomerulonephritis, PIGN and MGRS should be ruled out. If C3 glomerulopathy is diagnosed, a battery of complement testing for biomarkers, acquired drivers, functional assays, and genetic abnormalities is indicated. Results help to create an individualized treatment plan. Angiotensin-converting enzyme (ACE) inhibitors and/or ARBs are first line therapy for proteinuria and reduce blood pressure. Persistent proteinuria warrants a trial with mycophenolate mofetil (MMF) and if needed, a short course of steroids. If renal function continues to deteriorate, clinicians should encourage patients to consider the many ongoing clinical trials evaluating novel anti-complement therapies (see ClinicalTrials.gov). If terminal pathway activity is identified, eculizumab can be considered

should prevent C5 convertase formation and preventing formation of both convertases should provide disease-specific treatment for C3G. Within the next 1–2 years, it is very likely that one or more of these drugs will be available to the practicing nephrologist.

Each of the above targets is predicted to halt the amplification phase of complement activity, an important point when these drugs are compared to eculizumab and ravulizumab, both of which target C5 to prevent propagation of the terminal pathway of complement without affecting up-stream C3 convertase activity. Eculizumab is currently available to treat C3G on an off-label basis and is often considered when proteinuria and disease progression are refractory to immunosuppressive treatment with MMF. Eculizumab is most effective in patients with rapidly progressive C3G and terminal pathway activation. It is estimated that

of C3G patients treated with Eculizumab, ~10–20% will have a global response, ~20–25% will have a partial response, and ~55–70% will have no response to treatment (Le Quintrec et al., 2018; Ruggenenti et al., 2019).

4.3 | Transplantation

Data on transplantation outcomes are sparse making treatment decisions difficult. The challenge is highlighted by Angelo et al., who report results on 189,211 primary kidney transplants in the United Network for Organ Sharing (UNOS) database over the 20-year period from 1987 to 2007. Although C3G is not included, an omission reflecting the adoption of this term in 2013, MPGN type 2, an earlier classification for DDD, is found (Angelo, Bell, & Braun, 2011). Noteworthy is the rarity of this diagnosis (0.03% in the US ESRD population), the average time from diagnosis to ESRD (~10 years), and a poor allograft survival rate (Smith et al., 2019). When comparing recent studies, time to disease recurrence is ~11 weeks in DDD and ~19 weeks in C3GN. Graft loss is seen in ~70% of DDD and 30% of C3GN patients, with an average time to graft loss of ~43 months (Kumar et al., 2021; Regunathan-Shenk et al., 2019). Age at transplant also impacts 10-year allograft survival rates, which is 11% (8 of 72) for pediatric patients and 20.6% (22 of 107) for adults (Angelo et al., 2011).

More granular data are available from the study by Zand et al. in which they report outcomes in 21 patients (Zand et al., 2014). Median age at diagnosis was 20.8 years (based on the native kidney biopsy) with a median time to ESRD of ~3.5 years. Most patients opted for renal replacement therapy prior to transplantation and so the median age at transplantation was 36 years. Fourteen patients (67%) developed recurrent C3GN within 2–3 years of transplantation. In 10 cases, recurrence was suspected on clinical grounds, triggering the confirmatory biopsy. In four cases, however, histologic recurrence was recognized on a protocol biopsy prior to any evidence of clinical disease. Once recurrence was documented, median time to graft failure was 18 months.

A comparison of the recurrent to the nonrecurrent group was remarkable in three ways. First, there were more males than females in the nonrecurrent group (six males and one female versus six males and eight females; $p = .06$). Second, the HLA-DR17 (3)-DQ2 haplotype, which is seen in other autoimmune disorders like type 1 diabetes mellitus, was carried by 6 of 14 (43%) persons in the recurrent group versus only 1 of 7 (14%) persons in the nonrecurrent group. Since ~25% of the Caucasian population carries this haplotype; however, the significance of this finding is unclear (Burdett et al., 2003). Third, and perhaps most important, serum C3 levels differed between groups. Eight persons in the recurrent group had C3 measured pre-transplantation and in six, levels were very low (median, 33 mg/dl; NL 75–175 mg/dl). In contrast, in all six persons in the nonrecurrent group in whom C3 was measured, levels were normal. These complement results suggest that ongoing complement activity may be associated with disease recurrence in the allograft.

The risk of recurrent disease after kidney transplantation is impacted by underlying genetic abnormalities. Genetic variants in complement proteins are identified in ~30–40% of C3G patients; however, most of the identified variants are classified as variants of uncertain significance using criteria developed by the American College of Medical Genetics and Genomics (ACMG). Recently, Ren et al. evaluated the clinicopathologic significance of genetic variants in a TMA and C3G kidney transplant cohort. The group reported 10 variants in *CFH*, of which were four pathogenic, one was likely benign, and five were classified as variants of uncertain significance (Ren, Perkins, Love-Gregory, Atkinson, & Java, 2021). The presence of pathogenic variants increased the risk for recurrent disease in the transplant due to continued complement dysregulation in the recipient. In a study by Frangou et al., of 17 transplanted patients with CFHR5 nephropathy, a form of C3GN caused by an internal duplication of exons 2–3 within *CFHR5*, five patients lost their grafts due to disease recurrence, and in the remaining patients, the recurrence rate was 50% (Frangou et al., 2019). Graft loss with confirmed recurrence of CFHR5 nephropathy was likely in 62% of patients. These studies highlight the importance of genetic testing in C3G patients to identify genetic factors that may impact the risk of disease recurrence when considering transplantation.

To determine the efficacy of treatment on C3G recurrence in allografts, Suarez et al. pooled 12 studies with 122 C3G patients. Patients with significant proteinuria or acute kidney injury were treated for disease recurrence. Using a pooled heterogeneity model, the estimated rate of allograft lost was 81% after rituximab ($n = \sim 6$ of 7), 56% after therapeutic plasma exchange ($n = \sim 5$ of 10), and 22% after eculizumab ($n = \sim 7$ of 21; Gonzalez Suarez et al., 2020; Regunathan-Shenk et al., 2019). Pooled estimated rates of allograft loss in 66 patients who did not receive treatment were 53% and 32% for DDD ($n = \sim 15$ of 28) and C3GN ($n = \sim 9$ of 38), respectively. These results attest to the challenges in treating recurrence in the absence of disease-specific therapy.

5 | CONCLUSION

C3G is a pathologic description that defines a group of rare renal diseases characterized by complement dysregulation in the glomerular microenvironment often with concomitant complement dysregulation that can be quantitated in the circulation. The disease is often caused by immunological and/or genetic factors, although in a significant number of patients, current tests are unable to identify disease drivers. Treatment is unsatisfactory, and as a consequence, ~30–50% of patients progress to ESRD within 10 years of diagnosis. If transplantation is offered, allograft loss commonly occurs. Ongoing trials with new anti-complement drugs will hopefully offer a brighter future to persons diagnosed with this disease.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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