

In Search of C3G Tissue Biomarkers



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Complement (C) 3 glomerulopathy (C3G) is a rare, complement-mediated disorder that encompasses C3 glomerulonephritis (C3GN) and dense deposit disease (DDD). Drivers can be acquired autoantibodies and/or genetic mutations in distinct components and regulatory factors of the complement alternative pathway (CAP; Figure 1). Most common are autoantibodies, such as the C3 nephritic factors (detectable in 50%–80% of patients), which stabilize C3 convertase (C3bBb) to prolong its half-life, thereby promoting consumption of serum C3.¹ Approximately 50% of patients harbor autoantibodies to the C5 convertase (C3bBbC3b). Genetic factors, such as mutations in C3, complement factor H (fH), complement factor I (fI), complement factor B (fB), and complement factor-H-related protein (FHR) 5, have been identified in under 25% of C3G patients tested.¹ The *CFHR* family includes 5 closely related genes sharing short consensus repeats that are prone to copy number variations and rearrangements, further confounding genetic complexity.²

Although CAP dysregulation mediates C3G, it is unclear to what extent CAP dysregulation occurs in the fluid phase versus the glomerular microenvironment, including the endothelial glycocalyx and extracellular matrix components.¹ Activation in the fluid phase could shower the glomerular filtration barrier with complement debris. Once the debris is ensnared in the glomerular mesangium and capillary walls, local CAP activation might proceed unchecked, where release of anaphylatoxin C3a and chemotactin C5a promote leukocyte infiltration and cytokine-mediated glomerular injury. We know surprisingly little about which complement components accumulate in the glomerulus in C3G and how their relative abundance correlates with disease activity and outcome. A groundbreaking advance was the application of proteomics using laser capture microdissection and mass spectrometry to demonstrate a large array of CAP components in C3G glomeruli.³ C3 was the most abundant protein detected, followed in descending order of protein spectral counts by FHR1, C9, C5, FHR5, C8, C6, C7, FHR2, fH, and fI. Because the technique requires trypsin digestion, initial studies were unable to determine whether

the C3 spectra detected represented C3b or its breakdown products iC3b, C3c, and C3dg.³ In later studies, Sethi *et al.*⁴ showed that C3dg is the predominant cleavage product deposited, with similar peptide profiles found in C3GN and DDD.

Renal pathologists typically screen for C1q and C3c (a stable C3 cleavage product) in their routine panel of antibodies to diagnose glomerular diseases. For C3G, this approach is woefully insufficient to elucidate the “full complement” of complement proteins deposited in diseased glomeruli. In this issue, Medjeral-Thomas *et al.*⁵ applied a large battery of antibodies to individual complement factors and their regulatory proteins (including FHR5, FHR1, fH, C3b/iC3b/C3c, C3dg, C5b9, C1q, C4d, and properdin) to formalin-fixed paraffin-embedded (FFPE) biopsy samples from 19 patients with C3G (including 13 with C3GN and 6 with DDD). They catalogued the spectrum of immunohistochemistry results and made correlations with clinical and outcome parameters. Their findings provide the largest such analysis of C3G to date. Interestingly, the authors found a similar array of complement proteins as described by mass spectrometry, with comparable results in C3GN and DDD. A novel finding was the high prevalence of FHR5 in C3G glomeruli, where the intensity of staining correlated positively with the presence of a membranoproliferative pattern and with the staining intensities of C3 activation products (C3b/iC3b/C3c and C3dg) and C5b-9, and correlated negatively with estimated glomerular filtration rate at biopsy.⁵ These results suggest potential mechanistic relevance. FHR5 has been shown to activate CAP by (i) binding to extracellular matrix, where it directly promotes C3 convertase formation and C3b generation; and

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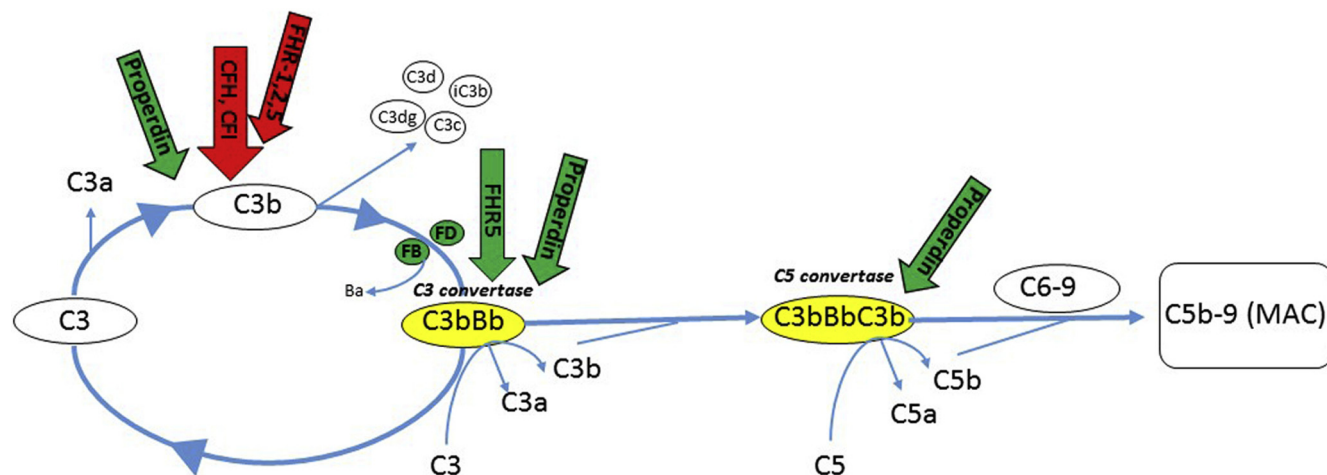


Figure 1. Overview of the components and regulatory factors in the complement alternative pathway (CAP). Alternative pathway dysregulation in complement (C)3G is characterized by inappropriate and excessive C3 consumption, C3 fragment production, and the subsequent deposition in the glomerular basement membrane and mesangium, causing glomerular injury. Dysregulation can stem from overactivity of CAP promoters (green) or loss of function/inhibition of CAP regulators (red). Complement factor H (CFH) is a major negative regulator of CAP via limitation of the amplification of C3b. CFH-related protein 5 (FHR5) has several actions that together activate the CAP: FHR5 competes with CFH for ligand binding to C3b, thereby antagonizing the ability of factor H to limit the amplification of C3b (a process called factor H deregulation). FHR5 also binds extracellular matrix components to support the assembly of the alternative pathway C3 convertase. CFI, complement factor I; FB, factor B; FD, factor D; FHR-1, complement factor H-related protein 1; FHR-2, complement factor H-related protein 2; MAC, membrane attack complex.

(ii) competing with fH for surface ligand binding, thereby reducing the ability of fH to negatively regulate surface C3b activation (a process known as fH deregulation).²

The use of immunohistochemistry on FFPE samples posed a number of technical challenges. First, the authors had to validate the ligand specificity of their antibodies on purified human proteins using enzyme-linked immunosorbent assays. Because many labs, particularly in the U.S., use immunofluorescence techniques on frozen tissue rather than immunohistochemistry on FFPE samples, the generalizability of the findings to frozen tissue needs validation. Prior direct comparisons between immunohistochemistry on FFPE samples and immunofluorescence on frozen sections have suggested that it is harder to quantitate staining intensity by immunohistochemistry, and even the staining results for C3c used to diagnose C3G are not always comparable.⁶ Use of random, archived FFPE sections precluded comparison of the intensity and distribution of staining

in a given glomerulus across serial sections, as would be needed to control for interglomerular heterogeneity and to compensate for the lack of systematic co-localization studies.⁵ Another potential confounder is the nonspecificity of FHR5 for C3G. FHR5 staining has been identified in glomeruli of diabetic nephropathy, IgA nephropathy, and membranous nephropathy, as well as sclerosing glomeruli irrespective of etiology.^{5,7} Thus, larger datasets of complement-mediated diseases and appropriate controls will be needed before conclusions can be drawn about the sensitivity of FHR5 as a marker of C3G.

The current criteria for diagnosis of C3G by immunofluorescence (dominant glomerular staining for C3 of at least 2 orders of magnitude greater intensity than for any other immune reactant)⁸ were developed at Columbia University to optimize sensitivity over specificity (i.e., avoiding false negatives and accepting false positives) because the diagnosis is intended to trigger a work-up of CAP dysfunction.

Cases of infection-related GN and immune complex-mediated membranoproliferative GN can meet immunofluorescence criteria for C3G, and repeat biopsies on some patients have shown a transformation from C3-dominant forms of GN to C3-only forms or immune complex-mediated forms.⁸ Sethi *et al.*⁹ have proposed that C4d staining equal to or more intense than C3 can serve as a positive marker for immune complex-mediated GN and, in contrast, absent or minimally detected C4d can serve as confirmation of C3G. The current study from Medjeral-Thomas *et al.*⁵ suggests that FHR5 staining may be a more suitable marker to confirm C3G than the absence of C4d because FHR5 was detectable in all their transplant biopsies of recurrent disease and all but one of their native biopsies, whereas up to 25% of their C3G biopsies demonstrated 3+ staining for C4d. Among the wide panel of complement proteins studied, FHR5 emerged as the most prevalent protein detected, at 3+ intensity,

outperforming glomerular C3 staining, the current hallmark of disease identification. Perhaps the most impressive diagnostic performance metric by FHR5 in this series is the single case of a protocol transplant biopsy in which glomerular FHR5 deposition was detectable in the absence of C3 staining, with biopsy-proven C3G recurrence seen in a subsequent for-cause biopsy 9 months later.

Beyond confirming diagnosis, however, lies the potential promise of FHR5 staining as a biomarker of disease activity and severity. The natural history of C3G does not follow a “one size fits all” pattern but displays a marked heterogeneity of disease course. Some patients maintain preserved renal function with hematuria and proteinuria for decades, whereas others rapidly progress to end-stage kidney disease within 1 year of diagnosis. To date, the best prognostic markers for progression have been presenting renal function and degree of chronicity on biopsy,^{1,7} which are not disease-specific for C3G. Our group has created a histopathologic index to score C3G biopsies,¹ and both the activity and chronicity scores showed significant association with progression to end-stage kidney disease; still, the components of this score are not specific to C3G. Attempts to correlate complement activation—via serum C3, C4, and/or hemolytic complement 50 (CH50) levels—with outcomes have not been successful, and the presence or absence of identifiable CAP abnormalities (i.e., mutations and/or autoantibodies) have been linked only inconsistently to rates of disease progression. Ongoing work to identify novel serum biomarkers (e.g., factor B split products, which denote newly formed C3 convertase, and C3a, a potential readout of alternative pathway C3

convertase activity) remains in the early stages and may be confounded by therapy exposure. A biopsy-based biomarker that connotes high risk for progression, obtained at the time of C3G diagnosis and easily accessible to diagnostic renal pathology laboratories, could facilitate early entry into complement-targeting clinical trials and potentially obviate exposure to immunosuppression in those patients with low-intensity staining. Medjeral-Thomas *et al.*⁵ showed a correlation between FHR5 and disease severity (via estimated glomerular filtration rate) at the time of diagnosis, but what they did not report is whether FHR5 intensity independently correlated with progression to end-stage kidney disease, which occurred in 48% of their cohort.

Indeed, many of the next steps needed to evaluate the role of FHR5 immunostaining in the management of C3G patients have been set forth by the authors of this interesting but small and retrospective study. Expanding the number of C3G patient samples, including native and allograft kidneys (both protocol and for-cause biopsies), will be important, along with the linkage between biopsy findings and long-term outcomes of progression to end-stage kidney disease. To that end, the assembled cohort ideally should include a broad spectrum of disease course; conceivably, the findings in this particular study were influenced by disease severity among the patients used for analysis, of whom nearly half progressed to end-stage kidney disease within a median of 40 months. Finally, via serial biopsies, it is important to demonstrate how FHR5 staining evolves in patients who have experienced some form of disease remission, clinically and histopathologically, as a true disease

biomarker would be expected to modulate alongside reductions in proteinuria, serum creatinine level, and glomerular proliferation.

DISCLOSURE

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