C3 Glomerulopathy: The Genetic and Clinical Findings in Dense Deposit Disease and C3 Glomerulonephritis

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C3 glomerulopathy (C3G) describes a group of very rare renal diseases in which dysregulation of the alternative and terminal complement pathways plays a pivotal pathogenic role. Dysregulation is driven by genetic and/or acquired defects, with interindividual variability giving rise to two broad subtypes of C3G—dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). Patient evaluation should include genetic testing and biomarker profiling of complement activity. There is currently no effective targeted treatment option for C3G and, as a consequence, a variety of supportive measures are used. C3G remains an ideal disease in which new complement therapies can be tested as they become available. Trials must include a comprehensive evaluation of each patient at the genetic and biomarker level so that individual responses to therapy can be predicted and understood in light of the degree of complement dysregulation and underlying pathology.

Abstract

C3 glomerulopathy (C3G) defines a group of very rare renal diseases characterized by complement C3 accumulation in renal glomeruli. Traditionally, this was in the setting of the absence or near absence of immunoglobulins. However, this criterion was over-stringent and recently it has been suggested that C3G is more appropriately identified by selecting glomerular changes in which there is “C3 dominant” staining. C3 dominant is defined as C3 intensity at least two orders of magnitude greater than any other immune reactant.1 The two major subgroups of C3G—dense deposit disease (DDD) and C3 glomerulonephritis (C3GN)—are resolved by electron microscopy (EM). DDD is defined by the EM findings of intramembranous glomerular basement membrane dense deposits, and C3GN encompasses the remainder of the C3G lesions and is defined by some combination of mesangial, subepithelial, subendothelial, and/or less dense, discontinuous intramembranous deposits.2

Clinically, C3G presents with proteinuria, hematuria, and often some degree of renal failure,3–6 although in DDD, acquired partial lipodystrophy and ocular drusen may be seen in addition.3 The annual incidence of biopsy-proven disease is 1 to 2 per million,4 with both sexes affected equally.4,7 Median age at C3G diagnosis as reported by Medjeral-Thomas et al is 21 years,4 although DDD typically presents earlier (mean age at diagnosis, 14 years8; however, one-fifth of DDD patients are older than 60 years7). Ten-year progression to end stage renal failure (ESRF) is ~30–50%,4 although among DDD patients it appears higher (progression to ESRF in DDD, 36–50% vs. ~25% in C3GN8). Recurrence of disease and allograft loss after transplantation is common (50–75%8).
C3G is caused by genetic or acquired dysregulation of the alternative pathway (AP) of complement. Examples of the former include the report by Gale et al\(^9,10\) of a genomic rearrangement in Cypriots in which exons 2 and 3 of the complement factor H-related 5 gene (CFHR5) are repeated resulting in a longer translated protein (SCRs 1 and 2 are duplicated) (\textit{►} Fig. 1). The phenotypic consequence is a variably penetrant dominant disease. Eighty-two of 91 patients (90%) carrying the novel CFHR5 gene presented with microscopic hematuria, and of these patients, 31 of 82 (38%) also had proteinuria. Twenty-eight proteinuric patients (90%) developed chronic renal failure (CRF). Time with disease was a significant contributor to ESRF—80% of men (16 of 20) and 21% of women (5 of 23) older than 50 years progressed to ESRF, with the reason for the male preponderance remaining unclear. The biopsy in all cases was consistent with C3GN.

In a second noteworthy report, Martínez-Barricarte and colleagues described a woman and her identical twin sons who segregated a two amino-acid deletion in C3 (C3\(_{923}\Delta DG\)) that results in a DDD phenotype.\(^11\) The mutant protein was the predominant C3 plasma protein. It circulated as non-activated C3 and C3\(_{923}\Delta DG\) convertase that was resistant to regulation by complement factor H. The net consequence was uncontrolled mutant fluid-phase C3 convertase (C3\(_{923}\Delta DG\) convertase) activity. However, the accelerated decay of the C3\(_{923}\Delta DG\) convertase by decay accelerating factor (DAF, CD55), and membrane cofactor protein (MCP, CD46) cofactor activity for complement factor I (CFI) were unaffected. As a result, cell-surface control of the AP was not impaired. The mother and one son were dialysis dependent.

Studies using laser capture and mass spectrometry of the glomeruli in sporadic cases of DDD\(^12\) and C3GN\(^13\) are consistent with the insights the above families have provided to our understanding of the disease process driving C3G. In both diseases, C3b, inactive C3b, and other alternative and terminal complement pathway proteins are found in glomeruli further linking complement dysregulation to C3G. Since understanding complement biology is a prerequisite for understanding C3G pathophysiology, we will briefly overview complement biology.

**The Complement Cascade**

The complement system is the cornerstone of innate immunity. It serves as the first line of defense against invading microorganisms, which can be lysed through the generation of membrane attack complex (MAC) or opsonized by C3b and C4b and marked for phagocytosis. C3b and C4b also play an important role in solubilizing and removing immune complexes from the circulation. Three components of complement—C3a, C4a, and C5a—are inflammatory activators and induce vascular permeability and the recruitment and activation of phagocytes.

Complement activity in the initiation phase is triggered through three pathways—the classic (CP), lectin (LP), and alternative (AP) (\textit{►} Fig. 2). While the CP is activated by immune complexes containing IgG or IgM as well as some microorganisms\(^14\) and the LP is activated by binding to carbohydrates on the surface of a wide range of pathogens\(^14\) (viruses, bacteria, fungi, protozoa), the AP is constitutively active through a process known as “tick-over.”\(^15\) “Tick-over”
occurs because C3 has a reactive thiol ester which spontaneously hydrolyzes to C3(H2O). The hydrolyzed C3(H2O) is able to interact with factor B that is then cleaved by factor D to generate a C3 convertase: C3(H2O)Bb. This enables direct cleavage of C3 to C3b, which, unless regulated, can rapidly amplify through a positive feedback loop. Binding of C3b to C3 convertase produces C5 convertase (C3bBbC3b), which then triggers the formation of the terminal complement complex and the anaphylatoxin, C5a.

Close regulation of the complement cascade is important to homeostasis and health, and is provided in the fluid-phase by proteins like complement factor H (CFH) and on cell surfaces by DAF and MCP. An imbalance in activity versus control leads to disease. For example, the Cypriot CFHR5 nephropathy described earlier is the result of loss of complement regulatory function in the glomerular microenvironment as opposed to more global systemic activation of complement in the fluid phase since C3 serum levels are normal in these patients. The C3G223DG mutation, in contrast, results in enhanced activity and uncontrolled fluid-phase activity of the mutant C3 convertase. In both cases, however, the consequence of complement dysregulation is C3G.

Genetic and Acquired Factors in DDD and C3GN

Familial cases of DDD and C3GN have provided important insight into disease pathogenesis. In addition to the reports by Gale et al and Martínez-Barricarte et al, familial C3GN has been reported in association with a hybrid CFHR3–1 protein, an internal duplication of CFHR1, and an internal duplication in CFHR5 in a family without Cypriot ancestry. DDD has recently been linked to a fusion protein consisting of...
**CFHR2** SCRs 1 and 2 coupled to **CFHR5**. These changes are shown schematically in – Fig. 1.

The impact of these rearrangements is clearer, at least on a structural basis, now that it has been shown that CFHR1, 2, or 5 interact with each other. The first two SCR domains of these three proteins are almost identical and form a tight head-tail dimer. This association enables homodimers (R1–R1, R2–R2, R5–R5) and heterodimers (R1–R2, R1–R5, R2–R5) to form. As CFHR1 is the most abundant protein, the predominant species are likely to be those that contain CFHR1 (R1–R1, R1–R5, R1–R2). Furthermore, in plasma these proteins form larger oligomeric complexes. Although the structural basis of dimerization has been resolved using X-ray crystallography, the structural basis of the oligomeric species is not defined. These findings suggest that the abnormal CFHR proteins associated with C3G may promote formation of unusual dimers and/or multimers that impact control of the C3 and/or C5 convertases in the fluid phase or in the microenvironment of the glomerulus (– Fig. 3).

Familial cases justify genetic testing in sporadic cases. To date, 20 different mutations have been identified in 12 different genes, with the **CFH-CFHR** gene family and **C3** implicated in 50% and 15% of cases, respectively (– Table 1). What is more noteworthy, however, is the very strong association of C3G with specific alleles of several genes that define disease haplotypes or complotypes. For example, in DDD the C3 at-risk haplotype is **GGA** (haplotype 2, defined by -311 T > C [rs3753394], p.V62I G > A [rs800292], p.Y402H T > C [rs1061170], and p.Q673 A > G [rs3753396]). In C3GN, the haplotype **MCPaaggt** (652 A > G [rs2796267], 366 A > G [rs2796268], IVS9–78 G > A [rs1962149], IVS12 + 638 G > A [rs859705], c.4070 T > C [rs7144]) defines the at-risk allele (– Fig. 4).

Most DDD patients (80–85%) and many C3GN patients (~50%) also develop autoantibodies to C3 convertase called C3 nephritic factors (C3Nefs), which protect the convertase against CFH-mediated decay, prolonging its half-life and leading to fluid-phase dysregulation of the AP. Autoantibodies against other proteins such as **CFB** are occasionally detected as well. Whether or how the C3G complotypes shown in – Fig. 4 contribute to or facilitate the development of autoantibodies is not known.

**Understanding the Differences between DDD and C3GN**

Although DDD and C3GN are both subtypes of C3G, they are differentiated histologically by their EM differences and clinically by the generally more aggressive course of DDD. Although there are no clear boundaries distinguishing these two diseases, considerable research is being done to understand the pathophysiological reasons for the observed differences. Using serological biomarkers of complement activity, recent research has shown that in these two diseases both the AP and terminal complement pathway...
are overactive as compared with controls (Fig. 2). In general, biomarker profiling also shows that dysregulation of the AP is greater in DDD than in C3GN, while for the terminal complement pathway, the reverse is true. However, our current composite understanding of C3G based on genetic studies and biomarker profiling is incomplete. We do not know how the unique microenvironment of the glomerulus contributes to disease outcome and neither do we know the role played by the kidney itself in either contributing to or protecting against disease.

**Treatment**

There is currently no broadly effective targeted treatment option for C3G and, as a consequence, a variety of supportive measures have been used. In a French cohort, treatment with angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) has shown to improve renal survival ($p < 0.0001$). Although plasma exchange should theoretically be useful as it can remove autoantibodies and mutated proteins, it has met with only limited success. In an early report of a 15-year-old girl with C3Nefs, plasma exchange was used to remove these autoantibodies from the circulation when DDD recurred in her allograft. Unfortunately, treatment was eventually discontinued as continual thrice weekly plasma exchange was required to keep C3Nefs levels down and that was not sustainable. The use of immune suppression in a French cohort also did not change renal survival. In aggregate, these data support the genetic and clinical findings of C3 glomerulopathy by limiting the effects of anaphylatoxins (C3a and C5a), inhibiting immune cell reaction and inflammation, and reducing antibody production, several case reports suggest otherwise.

Table 1 Gene variants reported to be associated with C3G

<table>
<thead>
<tr>
<th>Gene</th>
<th>HMD amino acid change</th>
<th>HGVS nucleotide</th>
<th>HGVS protein</th>
<th>Phenotype</th>
<th>References</th>
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<tr>
<td>C3AR1</td>
<td>Leu84Phe</td>
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<td>p.L84F</td>
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<td></td>
<td>c.2768_2773delACGGTG</td>
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<td>CFD</td>
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<td>DDD</td>
<td>Westra et al26 (2011)</td>
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<td></td>
<td>c.2171delC</td>
<td></td>
<td>C3GN</td>
<td>Sethi et al29 (2012)</td>
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*ADAM19 (ADAM metallopeptidase domain 19 (meltrin-β) NM_032774.3); C3AR1 (complement component 3a receptor 1, NM_000454.2); C3 (complement component 3, NM_000064.2); C8A (complement component 8, α-polypeptide NM_000562.2); CFD (complement factor D (adipsin), NM_001928.2); CFH (complement factor H (HF1), NM_000186.3); CFHR3 (complement factor H-related 3, NM_021023.5); CFHR1 (complement factor H-related 1, NM_002113.2); CFHR5 (complement factor H-related 5, NM_030787.3); CI (complement component 3a receptor 1 NM_000651.4); MCP (also known as CD46, membrane cofactor protein NM_002389.4). HGMD, Human Gene Mutation Database.

HGMDprofessional 2013 [accessed on December 9, 2013]; HGVS, nomenclature created by the Human Genome Variation Society to unequivocally describe sequence variants.

C3G, C3 glomerulopathy; C3GN, C3 glomerulonephritis; DDD, dense deposit disease.
atypical hemolytic uremic syndrome, attention has naturally focused on C3G. Eculizumab is a humanized monoclonal antibody against C5 which prevents activation of the terminal complement pathway and formation of MAC. It has met with mixed results in its use in C3G. In the largest single study to date—an open-label, proof-of-concept, efficacy-and-safety study of three patients with DDD and three patients with C3GN—improvements in either serum creatinine or histology were observed in four patients. In the remaining two patients, renal function declined during treatment. Based on the pathophysiology of C3G, this type of mixed response would be expected. In patients with significant complement dysregulation at the level of the C3 convertase, which is upstream of the point of action of eculizumab, although eculizumab therapy might conceivably slow down disease progression (by preventing C5a generation and/or MAC), it would not be curative.

C3G will be an ideal disease in which new complement therapies can be tested as they become available. However, because C3G is complicated, a comprehensive evaluation of each patient at the genetic and biomarker level must be completed so that individual responses to therapy can be predicted and understood in light of the degree of complement dysregulation and underlying pathology.

Concluding Remarks

C3G describes a group of very rare renal diseases in which dysregulation of the AP and terminal complement pathway plays a pivotal pathogenic role. Dysregulation is driven by genetic and/or acquired defects with a great deal of interindividual variability. Although we now understand a great deal of the overall disease process, more comprehensive genetic and biomarker studies are needed to refine our knowledge base. These studies will allow us to offer patients well-planned and well-designed clinical studies as new anti-complement agents are developed.

References


Mayilyan KR. Complement genetics, deficiencies, and disease associations. Protein Cell 2012;3(7):487–496


