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Novel Biomarkers in Glomerular Disease

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Abstract

Glomerular diseases are major contributors to the global burden of end stage kidney disease. The clinical course and outcome of these disorders are extremely variable and difficult to predict. The clinical trajectories range from a benign and spontaneously remitting condition to a symptomatic and rapidly progressive disease. The diagnosis is based entirely on the evaluation of kidney biopsy, but this invasive procedure carries multiple risks and often fails to predict the clinical course or responsiveness to treatment. However, more recent advances in genetics and molecular biology facilitated elucidation of novel pathogenic mechanisms of these disorders. These discoveries fuel the development of novel biomarkers and offer prospects of non-invasive diagnosis and improved prognostication. Our review focuses on the most promising novel biomarkers that have recently emerged for the major types of glomerular diseases, including IgA nephropathy, membranous nephropathy, focal segmental glomerulosclerosis, and membranoproliferative glomerulonephritis.

Keywords

glomerular disease; biomarkers; IgA nephropathy; membranous nephropathy; focal segmental glomerulosclerosis; membranoproliferative glomerulonephritis

Glomerular diseases are an important group of kidney disorders and collectively represent a major cause of end stage renal disease (ESRD) worldwide. The presentation, clinical course, and outcome of glomerular diseases are highly variable. Some patients achieve complete spontaneous remission, while others progress rapidly to ESRD despite aggressive treatment. Although renal biopsy is the gold standard for the diagnosis and evaluation for treatment, it is invasive and has several serious complications. In many cases, histopathology is neither diagnostic nor prognostic and fails to predict response to therapy, likely because many

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Web Resources: IgAN Progression Calculator: http://www.columbiamedicine.org/divisions/gharavi/calc_progression.php

IgAN Genetic Risk Score Calculator: http://www.columbiamedicine.org/divisions/gharavi/calc_genetic.php

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heterogeneous pathogenetic mechanisms generate clinically indistinguishable histology. Thus, beyond histopathological evaluation, clinical biomarkers of specific pathogenic processes may improve sub-classification and facilitate therapeutic choices. This review provides an update on the most promising glomerular disease biomarkers tested in clinical studies of four major types of glomerular diseases: IgA nephropathy (IgAN), membranous nephropathy (MN), focal segmental glomerulosclerosis (FSGS) and membranoproliferative glomerulonephritis (MPGN).

IgA Nephropathy (IgAN)

IgAN is the most common form of primary glomerulonephritis. The clinical spectrum of IgAN covers a wide range of features, from minor urinary abnormalities to rapidly progressive renal failure. In general, approximately 20-40% of patients with IgAN develop ESRD within 20 years of diagnosis¹⁻³. The disease is characterized by mesangial deposition of polymeric IgA1. The diagnosis is based entirely on the examination of kidney biopsy tissue and is defined by dominant or co-dominant glomerular IgA staining. A large number of studies established that abnormal O-glycosylation of IgA1 represents one of the key pathogenic events in IgAN. The defective glycosylation pattern involves galactose deficiency in the side chains of O-linked glycans attached to the hinge region of IgA1 heavy chains. The galactose-deficient IgA1 (Gd-IgA1) is present in the mesangial immune deposit and its serum level is quantifiable by ELISA. However, high level of circulating Gd-IgA1 alone does not induce renal injury, and additional factors are likely required for the full expression of nephritis. Current data indicates that at least four hits may contribute to development of IgAN: aberrant glycosylation of IgA1 (hit #1), synthesis of antibodies directed against Gd-IgA1 (hit #2), formation of circulating immune complexes (hit #3), and deposition of these complexes in the glomerular mesangium (hit #4)⁴⁻⁶. The deposits promote glomerular inflammation, cause local activation of the complement system, and stimulate proliferation of mesangial cells. For more extensive overview, we refer the interested reader to this year's extensive reviews on the genetic⁵, immunologic⁷, and clinical⁶ aspects of this disease.

Clinical markers

Predictors of renal failure in IgAN have been assessed in several clinicopathologic studies; baseline clinical factors most consistently found to be independently associated with progressive renal disease include decreased renal function at the time of diagnosis⁸⁻¹¹, histologic grading⁸⁻¹² and proteinuria¹²⁻¹⁴. Some studies suggest additional predictive value of age¹¹, gender¹¹, high blood pressure at presentation⁵⁻⁷, serum albumin level^{11, 15}, hematuria¹¹, and family history^{9, 11}. More recently, a helpful clinical progression risk score has been proposed that estimates the probability of ESRD based on 4 clinical variables at the time of renal biopsy: eGFR, systolic blood pressure, hemoglobin and serum albumin level (see Web Resources for online calculator)¹⁶. Although this simple risk score outperformed previously proposed renal disease progression scores, it was designed based on a single Chinese cohort, and will require prospective validation in Caucasians.

Histopathologic markers

On biopsy, IgAN is characterized by focal mesangial proliferation and matrix expansion accompanied by mesangial deposits of IgA and often weaker staining for IgG, C3, and C5b-9. Although a number of histopathological classifications for IgAN have been proposed, older scoring systems had generally poor correlation with clinical outcomes. Recently, a new Oxford classification system has been proposed based on four histologic criteria that best correlate with disease progression: mesangial proliferation (M), endocapillary proliferation (E), segmental sclerosis (S), and tubular atrophy/interstitial

fibrosis (T) ¹⁷. In the validation studies, the T-score is most consistently associated with poor prognosis, while the E-score appears least reproducible ^{18, 19}. Although the Oxford scoring system does not include crescentic lesions, follow-up studies and clinical experience suggest that crescents represent an important prognostic factor ¹⁸ and the crescent (C) score may be included in the future modifications of this classification. In addition to the type of histologic lesions, tissue markers of complement activation, such as intensity and pattern of staining for C3, C4d, C5b-9 and MBL, may offer supplemental information about disease activity ²⁰⁻²².

Blood markers

The serum levels of Gd-IgA1 and anti-glycan antibodies directed against the hinge region of Gd-IgA1 represent the most promising candidate biomarkers for IgAN ^{23, 24}. A lectin-based ELISA assay for circulating Gd-IgA1 demonstrates 90% specificity and 76% sensitivity to diagnose IgAN, thus it appears to be one of the best candidates for a new non-invasive diagnostic test ²³. However, this test utilizes a naturally occurring lectin and has been difficult to standardize, thus has not yet been introduced in routine clinical practice. Utilizing this assay, a recent study from China suggested that high level of Gd-IgA1 at the time of diagnosis was associated with faster rate of renal function decline ²⁵. These observations are promising, but will require replication in independent cohorts. Interestingly, the glycosylation defects appear to have a strong genetic determination, with heritability of over 50% ^{26, 27}. In family-based studies, it became apparent that many of the asymptomatic relatives of IgAN cases have elevated Gd-IgA1 in the absence of kidney disease, suggesting that elevated Gd-IgA1 level alone is not sufficient to produce clinically significant disease. These observations suggest that additional factors (or “hits”) are required for full disease expression. The formation of anti-glycan antibodies directed at the hinge region of Gd-IgA1 emerged as one of the most likely candidates for a “second hit” ²⁴. The test for anti-glycan antibodies could be used in combination with Gd-IgA1 levels to identify patients at high risk of disease. Moreover, early studies indicate that elevated levels of antiglycan IgGs in the sera of patients with IgAN correlate well with proteinuria ²⁴ and may predict faster progression to ESRD ²⁸. More formal evaluation of the diagnostic and prognostic utility of Gd-IgA1 levels in combination with antiglycan antibody titers awaits well-designed clinical studies.

Urine markers

Presently, there are no reliable urinary markers with clinical utility in IgAN. Several candidates have been studied with variable success, including urinary levels of immune complexes containing Gd-IgA1 ²⁹ and urinary markers of complement activation, such as C5b-9, FH, MBL, and properdin levels ^{30, 31}. Newer approaches based on the analysis of urinary proteome offer prospects to identify novel candidate peptides ³²⁻³⁴, but applications to IgAN have been limited. Similarly, urinary RNA profiling, including microRNAs (short noncoding RNA molecules that regulate gene expression) may offer completely novel molecular markers ³⁵⁻³⁷, but initial studies in IgAN suffer from small sample sizes.

Genetic markers

Most cases of IgAN are sporadic, although familial forms are likely under-diagnosed and may account for up to 1-5% of cases. Although several linkage studies in familial disease have been performed, the identity of specific genes underlying familial IgAN remains unknown ³⁸. The genetic determinants of sporadic IgAN have been investigated using GWAS approach ^{26, 27, 39}. The strongest signal consistently observed in all IgAN GWA studies involves the MHC region on chromosome 6p21. This signal is complex and constitutes at least three distinct regions of association, including: (1) *HLA-DR* and *-DQ*

genes involved in the MHC class II response; (2) *TAP1/2* and *PSMB8/9* genes involved in antigen processing for presentation by MHC class I; and (3) *HLA-DP* genes also involved in class II response^{26,27}. Additional IgAN loci outside of the MHC region include: (1) a common deletion of *CFHR1* and *CFHR3* genes on chromosome 1q32; (2) chromosome 22q12 locus that contains several genes, including *HORMAD2*, *MTMR3*, *LIF* and *OSM*, and appears to control IgA levels; (3) another gene-rich region on chromosome 17p23 that contains *TNFSF13* gene and is also associated with IgA levels, and (4) a region on chromosome 8p23 in the cluster of *DEFA* genes. Cumulatively, these loci explain approximately 5% of variance in disease. A genetic risk score based on SNP genotypes at the above loci has been proposed^{26, 27} and implemented into a convenient online calculator (see Web Resources). This risk score has been demonstrated to correlate closely with disease prevalence across worldwide populations²⁷ and is expected to be refined with the newer wave of GWA studies. Taken together, GWA studies of IgAN have several important implications. First, GWAS results indicate that IgAN has a complex genetic architecture, with multiple loci individually exerting moderate to small effects on disease risk. Second, strong MHC signal and additional signals in the genes encoding immune-related molecules highlight the key role of antigen presentation, complement system, and mucosal immunity in the pathogenesis of IgAN. Third, the worldwide distribution of IgAN risk alleles correlates strongly with disease epidemiology. As the predictive value of the SNP-based genetic score improves by incorporation of findings from newer studies, it may offer a powerful tool for disease prediction. Additional studies correlating the GWAS-based risk score with the parameters of disease severity, tissue injury, and overall renal prognosis are underway.

Idiopathic Membranous Nephropathy (iMN)

iMN is a common cause of nephrotic syndrome in Caucasians. It is diagnosed by kidney biopsy, which demonstrates characteristic immune deposits between the lamina rara externa of the glomerular basement membrane and the podocyte. The available evidence indicates that the deposits are formed in situ, at the base of the podocyte processes, due to binding of circulating antibodies directed against podocyte antigens⁴⁰. The course of iMN is extremely variable, with 1/3 of patients undergoing spontaneous remission, 1/3 experiencing sustained symptoms, and 1/3 progressing to ESRD⁴¹. The treatment is controversial and response rates are highly variable, likely due to disease heterogeneity. There has been an extensive search for biomarkers to define patients who derive most benefit from more aggressive immunosuppression.

Clinical Markers

The severity of sustained proteinuria and remission of proteinuria during follow-up are the most powerful predictors of outcome in iMN^{42, 43}. A clinical progression score has been proposed by the Toronto group that incorporates baseline renal function and the maximal level of persistent proteinuria during follow-up in predicting progressors from non-progressors^{42, 43}. However, the parameter of persistent proteinuria requires indefinite longitudinal follow-up, thus limiting the utility of this score in a clinical setting. A further modification of the risk score that defines persistent proteinuria based on only initial 6 months of follow-up appears more useful⁴⁴, however, there is a need for a more accurate clinical prediction tool that could be implemented at the time of diagnosis rather than follow-up.

Histopathologic markers

Traditional histopathologic staging of membranous nephropathy has poor correlation with clinical outcomes⁴⁵. Additional stains for proteins up regulated in the process of fibrosis and inflammation have been studied^{46, 47}, but it is not clear if these markers provide

additional value beyond the standard scoring of tubulo-interstitial injury. Markers of complement activation, such as C4d staining, may be more reflective of disease activity⁴⁸. Finally, tissue staining for anti-PLA2R antibodies, perhaps in combination with a serum test for anti-PLA2R antibodies, may represent one of the most promising new tissue markers with diagnostic utility (see below)⁴⁹.

Blood markers

In 2009, *Beck et al.* identified the M-type phospholipase A2 receptor (PLA2R) as a target podocyte antigen triggering an antibody response in MN⁵⁰. PLA2R is a 180-KDa polypeptide with a long extracellular domain, consisting of a cysteine-rich head and fibronectin type II-like repeat domains and eight repeated carbohydrate-recognition domains, is synthesized by podocytes and expressed at the membrane surface. It is believed that PLA2R transmits intracellular signals after binding to one or more of the soluble A2 phospholipases^{50, 51}.

The levels of anti-PLA2R antibodies, primarily of the IgG4 subclass, are elevated in approximately 60%-70% of patients with iMN, and a clear correlation between the antibody titers and the clinical disease activity has been demonstrated^{50, 52}. In recipients of kidney transplant, the presence of anti-PLA2R antibodies could be used to diagnose relapsing MN⁵³. Treatment with rituximab has been shown to reduce the antibody titers as well as urine excretion of protein⁵⁴. These compelling findings may soon change the diagnostic algorithm in patients with nephrotic syndrome; positive anti-PLA2R antibody titers in the setting of classic nephrotic presentation may become sufficient to establish the diagnosis, thus obviating the need for a renal biopsy.

The correlation of serum PLA2R autoantibodies titers with disease activity, the antibody presence within tissue immune deposits, and the association of common genetic variants in PLA2R with the risk of MN (see genetic markers below) have all hinted at direct antibody-mediated pathogenicity. Nevertheless, several caveats need to be considered here. First, the experimental demonstration of pathogenicity has been challenging, largely due to the lack of convincing animal models, thus the ultimate proof of causality is still missing. Second, although anti-PLA2R antibodies were absent in patients with proteinuria due to MCD, FSGS, or IgAN, the overall numbers of disease controls tested in the literature are relatively small^{50, 55, 56} and more data on the specificity of the test are needed. Additional caveat relates to the fact that some seronegative patients have tissue immunodeposits that are positive for anti-PLA2R antibodies. Thus, tissue immunostaining for PLA2R antibodies may still be needed in the setting of a negative serum test⁴⁹. Finally, membranous nephropathy can also develop secondary to systemic autoimmune diseases (SLE), infections (hepatitis B), drugs (NSAIDs), and malignancies. In these conditions, the subepithelial deposits may arise from deposition of circulating immune complexes, or from binding of antibodies to antigens that are derived from the tumor and were planted within the basement membrane⁵⁷. Although most individuals with secondary forms of MN are negative for anti-PLA2R antibodies, positivity for anti-PLA2R antibodies has been reported in this setting, and may represent independent development of autoimmunity and systemic disease^{52, 55, 58}.

Urine markers

Urinary levels of a number of proteins have been studied in relationship to the progression of renal disease in iMN, including TGF- β , beta2-microglobulin, IgG, urinary complement levels, N-acetyl-beta-glucosaminidase and L-fatty acid binding protein⁵⁹⁻⁶⁶. Major caveats of these markers include relatively poor sensitivity and specificity, small size of cohorts investigated, and a lack of well-powered independent validation studies. Larger studies

using unbiased proteomic approaches are clearly needed to facilitate the identification of novel urinary markers in iMN.

Genetic markers

To date, there has been only a single GWAS published for iMN⁶⁷. This study, based on three relatively small European cohorts, discovered a significant association of the *PLA2R* locus, along with a very strong and highly significant peak in the MHC region centered over the *HLA-DQA1* gene. Both loci were subsequently replicated in independent cohorts⁶⁸. Most impressively, the effect sizes at both of these loci were unusually large for a GWAS. The disease risk was 20-fold higher in individuals homozygous for the risk SNP in *HLA-DQA1* and 4-fold higher for homozygotes at the *PLA2R1* locus. Such large effects are rarely observed for common alleles, suggesting that either balancing selection or more complex evolutionary forces maintain these alleles at high frequency in Europeans. Among individuals who carry risk alleles at both loci, 73% have anti-PLA2R antibodies and 75% express PLA2R in glomeruli. In contrast, individuals who carry protective genotypes of both genes have no detectable circulating or tissue anti-PLA2R antibodies⁶⁸. These findings provide strong independent support for the central role of autoimmune response directed against PLA2R in the pathogenesis of iMN. However, follow-up studies need to address the utility of genetic typing for these alleles in the diagnosis of iMN. Finally, additional efforts need to be directed to explore genotype correlations with histopathologic features, clinical course, and overall prognosis.

Focal segmental glomerulosclerosis (FSGS)

FSGS can occur as a primary disorder due to genetic mutations in specific podocyte proteins, secondary to a wide range of toxic and environmental exposures, or as a consequence of adaptive changes to reduced kidney mass⁶⁹⁻⁷¹. Similar to other glomerular diseases, the diagnosis of FSGS relies entirely on kidney biopsy. Distinguishing between primary and secondary forms is of paramount interest for the treatment and overall prognosis in this condition, since only nephrotic patients with primary forms that are not produced by rare genetic mutations in podocyte genes are good candidates for immunosuppressive treatments⁷². It is also important to point out that minimal change disease (MCD) and FSGS are seen as a continuum of the same pathogenic process by one part of the field, whereas the other part considers these conditions as two completely separate disease entities. In this review, we concentrate primarily on FSGS, but we will often refer to MCD considering that many clinical studies do not make a sharp distinction between the two conditions.

Clinical Markers

In idiopathic nephrotic syndrome caused by MCD or FSGS, the severity and persistence of proteinuria, and response to steroid treatment have been identified as the main long-term prognostic factors regardless of the histological substrate⁷²⁻⁷⁴. While patients with sub-nephrotic proteinuria have a relatively good prognosis, in cases with clinical nephrotic syndrome the outcomes greatly depend on the baseline renal function, magnitude of proteinuria, and the degree of tubulo-interstitial injury^{73, 75, 76}. Many observational studies have also demonstrated that remission of proteinuria, whether spontaneous or induced by therapy, is associated with a good outcome. The disease resistance to corticosteroids and immunosuppressive therapy is considered to be one of the strongest predictors of ESRD^{74, 77-79}. Unfortunately, there are no reliable clinical predictors of steroid responsiveness, and a trail of steroids is typically administered after ruling out secondary causes.

Histopathologic markers

Histologic variants of FSGS include perihilar, cellular, tip, collapsing, and not-otherwise-specified (NOS) patterns⁸⁰. These morphologic patterns apply to both, primary and secondary forms of FSGS. The collapsing pattern, which is characterized by increased proliferation of podocytes and loss of markers of mature podocytes, represents the most severe form of injury and is associated with more rapid progression to ESRD. Conversely, FSGS with glomerular tip lesion more closely resembles MCD with respect to clinical features and has more favorable prognosis⁸¹. In addition to the morphologic classifications, several tissue markers have been proposed to better differentiate primary FSGS from MCD, or secondary forms of FSGS, including the expression of activated $\beta 3$ integrin⁸², CD80 (B7-1)^{83,84} and α -dystroglycans^{85,86}. Some of these markers have already been used in assessing responsiveness to treatment. For example, *in vitro* incubation of cultured podocytes with serum of subjects with FSGS demonstrated reduced podocyte $\beta 3$ integrin activity after plasmapheresis⁸². Recent data indicate that the podocyte expression of CD80 (B7-1) may be particularly promising for guiding therapies. The induction of CD80 expression, which is absent in normal podocytes, reduces podocyte capacity to attach to the surrounding matrix through $\beta 1$ integrin, leading to foot processes detachment and proteinuria. These observations motivated a small trial of abatacept, an inhibitor of B7-1 that is approved for rheumatoid arthritis, in CD80+ FSGS⁸⁴. The treatment with abatacept induced remission in a patient with primary FSGS and in four patients with recurrent FSGS after kidney transplantation. Although these results are extremely promising and exemplify successful application of tissue biomarkers in tailoring therapies for FSGS, larger trials of abatacept in CD80+ proteinuric disease are clearly needed to validate these new findings.

Blood Markers

Several observations support the hypothesis that, at least in some cases, FSGS is caused by a circulating permeability factor capable of damaging podocytes. These observations include the absence of immune deposits in kidney tissue, frequent disease recurrence after kidney transplantation, responsiveness to plasmapheresis, and reports of nephrotic syndrome transmission from affected mothers to newborns⁸⁷⁻⁹¹. Previously proposed candidate molecules include hemopexin, podocyte-secreted angiopoietin-like-4, vascular endothelial growth factor and cardiotrophin-like cytokine-1⁹¹. More recently, Wei *et al.* reported soluble urokinase receptor (suPAR) as a new putative permeability factor in FSGS⁸². The suPAR molecule represents the soluble form of the receptor for urokinase plasminogen-type activator and appears to be elevated in approximately two-thirds of studied patients with primary FSGS. The suPAR protein has a molecular weight of 20-50kDa, similar to the size predicted for the hypothetical permeability factor described in previous studies⁹². The mechanisms of renal toxicity of suPAR were studied both *in vitro* and *in vivo*. At the experimental level, FSGS lesions have been induced in transgenic mice that overexpress suPAR⁸². Present data indicate that suPAR could act through binding to the podocyte $\beta 3$ integrin, one of the principal proteins that anchor podocytes to the glomerular basement membrane. The interaction of suPAR and $\beta 3$ integrin produces structural changes in podocytes, altering the permeability of the glomerular filtration membrane⁹³. However, the precise source of circulating suPAR and specific triggers to its synthesis are still unknown. Elevated levels of this molecule have been reported in patients with malignancy and in the course of HIV infection^{94,95}. In FSGS patients that receive a kidney transplant, elevated suPAR prior to transplantation is associated with increased risk of disease recurrence, and preliminary evidence suggests that treatment with plasmapheresis may be beneficial⁸². Some studies also suggest that high levels of suPAR may be predictive of response to immunosuppressive treatment^{93,96}. Finally, recent report suggests placental transmission of suPAR as a cause of transient proteinuria in an infant born to a mother with FSGS⁹⁷.

Although the measurement of serum suPAR levels represents a promising diagnostic test, several important caveats of this test need to be considered. First, serum suPAR level was elevated only in 55%–84% of studied patients with FSGS. The observation that up to 45% of patients have normal suPAR levels suggests that primary FSGS may be a heterogeneous disorder and, in these cases, the disease may be caused by a pathogenic mechanism completely unrelated to the suPAR pathway. Second, commercial ELISA test measures glycosylated forms of suPAR. Although this assay can differentiate FSGS from normal controls and disease controls with mild degree of CKD before ^{82, 93, 98} or after kidney transplantation ⁹⁹, the level alone may not be that informative in advanced CKD or ESRD, as non-specific accumulation of suPAR may occur ¹⁰⁰. Similarly, pro-inflammatory states, malignancies, and infections may induce higher levels of suPAR glycoforms in the absence of overt proteinuric disease ¹⁰¹. These observations led to the hypothesis that various isoforms of suPAR may have different effects on the activation of podocyte $\beta 3$ integrin; some suPAR molecules may perhaps be more pathogenic compared to other isoforms ^{102,103}. More studies are clearly needed to precisely understand the differences in pathogenic strengths of various isoforms. In summary, while growing evidence accumulates in support of the pathogenic role of suPAR in a sizeable subset of patients with FSGS, more specific assays may be needed before suPAR testing can be recommended for routine clinical use.

Urine markers

In general, urine biomarker studies in FSGS suffer from small sample size and lack of rigorous replication. There is some evidence that urine levels of CD80 may be useful in differentiating between MCD and FSGS ⁸³, but no clear association with response to treatment has been demonstrated. To date, none of the urine biomarkers studied have been introduced into clinical practice.

Genetic markers

Over the past decade, significant progress has been made in the discovery of genetic basis of familial forms of FSGS. A growing number of autosomal dominant and recessive syndromes are now recognized, most caused by mutations in the genes encoding proteins involved in podocyte structure, motility, or signaling (see Table 1 for a full list of genes). Among the most common inherited causes of familial disease are mutations in the *INF2* gene ¹⁰⁴. These mutations account for up to 9% of familial FSGS ¹⁰⁵, thus genetic testing for this gene is warranted in individuals with a family history indicative of dominant transmission. Cumulatively, however, Mendelian syndromes account for a relatively small proportion of FSGS. The contribution of genetic variation in podocyte genes is less clear for sporadic FSGS.

Among common genetic polymorphisms, two variants in *APOL1* emerged as a major genetic risk factor predisposing to FSGS in individuals of African ancestry ¹⁰⁶. The FSGS risk variants in *APOL1* are protective against *Trypanosoma brucei rhodesiense* infection, and therefore provide an evolutionary advantage in Africa where this parasite represents an endemic cause of sleeping sickness. Accordingly, balancing selection explains why these risk alleles are common in African-Americans, but absent from European chromosomes. The effect of *APOL1* genotypes on the clinical course of disease and responsiveness to treatment represents an area of active research.

Membranoproliferative Glomerulonephritis (MPGN)

MPGN denotes a general pattern of glomerular injury characterized by an increase in mesangial cellularity and matrix with thickening of glomerular capillary walls secondary to

subendothelial deposition of immune complexes and/or complement factors, cellular entrapment, and new basement membrane formation ¹⁰⁷. Idiopathic MPGN is a diagnosis of exclusion and a systematic approach to evaluation will often uncover a secondary cause, such as an infection, autoimmune disease, monoclonal gammopathy, neoplasia, or chronic thrombotic microangiopathy ¹⁰⁸. Recently, improved understanding of the role of the alternative complement pathway in MPGN has illuminated the field and led to a paradigm shift in the disease classification. MPGN has now been reclassified into immunoglobulin-mediated disease (driven primarily by the classical complement pathway), versus non-immunoglobulin-mediated disease (fueled by the alternative complement pathway overactivity) ¹⁰⁹.

Clinical markers

MPGN commonly presents with proteinuria, microhematuria, hypertension, and kidney dysfunction. Reduction in the serum concentration of complement components (C3 and/or C4) is commonly observed, but the definitive diagnosis requires a kidney biopsy. The evaluation of patients with immune-complex-associated MPGN focuses on identifying the underlying etiology, including infections, autoimmune diseases, and monoclonal gammopathies. The non-immune-complex mediated MPGN (a.k.a. C3 glomerulonephropathy, or C3GN) is diagnosed when tissue staining is positive for C3 deposits, but negative for immunoglobulins. In these cases, a thorough evaluation to detect abnormalities of the alternative pathway is indicated. This evaluation should include genetic testing, autoantibody testing, and assays of alternative pathway. In general, there is a paucity of large cohorts of MPGN patients that are correctly re-classified according to the new criteria and followed longitudinally. As a result, there is generally no data on useful clinical prognosticators

Histopathologic markers

Glomerular deposits of C3 without immunoglobulins represent the hallmark of alternative pathway dysregulation through inherited or acquired defects. These immunoglobulin-negative forms are referred to as C3 glomerulonephropathies, which encompasses both dense deposit disease (DDD) and C3 glomerulonephritis (C3GN). Although DDD and C3GN are morphologically distinguishable by the appearance and location of deposits on electron microscopy, these diseases are now considered as part the same etio-pathologic spectrum ¹⁰⁷⁻¹⁰⁹. However, adult patients with DDD appear to have more aggressive disease and worse outcomes compared to C3GN ^{110, 111}. Proteomic analysis of biopsy tissue from patients with DDD identified that deposits contained components of the terminal complement pathway along with apolipoprotein-E ¹¹². Apolipoprotein-E may potentially represent a novel disease biomarker, although it may not be entirely specific to DDD ^{113, 114}. In summary, distinguishing C3 glomerulonephropathy from immunoglobulin-mediated MPGN is the first step in guiding the work-up and treatment algorithms. Further differentiation between DDD and C3GN may help with long-term prognostication.

Blood markers

While the immune-complex-mediated forms of MPGN are characterized by variable depression in both C3 and C4 complement components (indicating activation of the classical pathway), C3 glomerulopathy is often marked by low C3 but relatively normal C4 levels (predominant activation of the alternative pathway). More specific assays of complement system activity are also available in the work-up of C3 glomerulonephropathies, including an alternative pathway functional assay and assays for circulating complement breakdown products such as C3a, C5a, and soluble form of membrane-attack complex (sMAC) ^{109, 115, 116}. Overactivity of the alternative pathway can also be caused by acquired autoantibodies that stabilize C3 convertase (for example, C3Nef and anti-CFB

autoantibodies) or block the action of pathway inhibitors (for example, anti-factor H and anti-factor I autoantibodies)^{108, 109, 115-119}. The first described and best known of such autoantibodies is C3Nef¹¹⁷⁻¹¹⁹ which directly stabilizes the C3 activating complex and prevents the inhibitory action of factor H. Effectively, C3Nef binding prolongs the half-life of C3 convertase leading to massive consumption of C3 due to tissue deposition¹²⁰. C3Nef have been detected in the serum of up to 40% of patients with C3GN. However, a fluctuation in C3Nef activity was noted in approximately one-third of the patients during follow-up¹¹⁰. Since C3Nef is also detectable in a fraction of healthy individuals¹²¹ and patients with other kidney diseases^{122, 123}, the specificity of C3Nef in the diagnosis of C3GN remains unclear. In addition, many patients with C3Nef will also have a second abnormality detected on screening, such as genetic mutation in factor H^{124, 125}, or additional auto-antibodies directed against factors H, I, or B¹²⁶⁻¹²⁸. Among patients with identifiable genetic defect in the complement pathway, over 50% also have detectable C3Nef¹¹⁰. The co-existence of inherited and acquired abnormalities of the alternative complement pathway raises the hypothesis that genetic hits may promote autoimmune phenomena. Presently, the exact mechanism underlying these observations remains unknown.

Urine markers

Well-designed urinary biomarker studies are still lacking in patients with MPGN.

Genetic markers

Familial studies and single case reports have provided unique insights into the heterogeneous features of C3GN^{124, 129}. Mutations in the regulators of the alternative pathway represent a common cause of C3 glomerulopathy, and comprehensive genetic screens should be performed as part of disease work-up. The principal pathway activators with reported mutations include *C3* and *CFB*. The key inhibitors for genetic screening include *CFH*, *CFI*, and *MCP*. In addition, genetic variants in any of the five factor-H-related proteins (*CFHRI-5*) are also occasionally involved, but because these genes are particularly prone to segmental deletions and duplications, more specialized molecular tests that assess copy number variation are required. In summary, as the catalogue of mutations in complement regulatory genes becomes more complete and their phenotypic consequences are better delineated, molecular diagnosis will undoubtedly represent the best way to risk stratify patients and personalize therapies. This approach is further facilitated by recent advances in NextGen sequencing technology, which allows for quick and cost-effective screening of a large number of genes based on a single blood test.

Conclusions

In conclusion, recent years have been marked by a dramatic progress in elucidating genes and specific molecular pathways involved in the pathogenesis of glomerular disorders. These developments have already accelerated biomarker discovery and offered novel candidate pathways for potential therapeutic interventions. Several new NIH-sponsored initiatives are directed at establishing well-powered longitudinal cohorts of glomerular disease patients with extensive archives of DNA samples, kidney tissue, blood and urine bio-specimens. These efforts include the Nephrotic Syndrome Study Network (NEPTUNE)¹³⁰, as well as the NIDDK's "Advancing Clinical Research in Primary Glomerular Diseases" project that aims to establish a longitudinal cohort of 2,400 patients with primary glomerular disease. Such large collaborative initiatives will greatly facilitate rigorous biomarker discovery studies, and are long overdue in the field of glomerular disease.

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Table 1
Most promising glomerular disease biomarkers

	IgAN	MN	FSGS	MPGN
Blood Markers	-Galactose-deficient IgA1 -Antiglycan antibodies -IL6	-Anti-PLA2R Ab -Anti-AR Ab -Anti-SOD2 Ab -Anti- α -enolase Ab -Anti-NEP Ab -Anti-bovine serum albumin Ab	-Soluble urokinase receptor (suPAR) -Podocyte-secreted angiopoietin-like-4 -CLC-1 -Hemopexin	-Complement assays: C3, C4, CH50, APFA, Soluble MAC, C3a, C5a. -C3Nef -Anti-factor B Ab -Anti-factor H Ab -Anti-factor I Ab
Histopathologic Markers	-MEST classification -Intensity of IgA stain -C3, C4d, MAC, MBL	-Staging I-V -Anti-PLA2R Ab -C4d -Interstitial SMA -MCP-1	-Columbia classification -CD80 - α -dystroglycans -Podocyte-secreted angiopoietin-like-4	-Classification based on immunoglobulin and C3 staining -DDD or C3GN pattern -Apolipoprotein-E
Urinary Markers	-MAC -factor H -Properdin -urinary MBL -microRNAs (miR-200, miR-205, miR-192, miR-29b, miR-29c and miR-93)	-IgG -TGF- β - β 2-microglobulin -C3, C4, C3dg, MAC - β -NAG -L-FABP -ua1m	-Urinary retinol-binding protein - α 1AT fragments -uCD80 -TGF- β -miR-192, miR-205	-uCD80 -MAC
Genetics- Common Variants (GWAS)	- <i>HLA-DQ/DR</i> locus - <i>HLA-DP</i> locus - <i>TAP1/PSMB8</i> locus - <i>HORMAD2</i> locus - <i>CFHR3,1</i> -deletion - <i>TNFSF13</i> locus - <i>DEFA</i> locus	- <i>PLA2R1</i> locus - <i>HLA-DQ/DR</i> locus	- <i>APOL1</i> locus (<i>G1/G2</i> variants)	-unknown
Genetics-Rare Variants (Mendelian subtypes)	-unknown	- <i>MME</i> (maternal mutations)	- <i>INF2</i> , <i>ACTN4</i> , <i>CD151</i> , <i>CD2AP</i> , <i>COQ2</i> , <i>COQ6</i> , <i>DGKE</i> , <i>IFN2</i> , <i>ITGB4</i> , <i>LAMB2</i> , <i>LMX1B</i> , <i>MYH9</i> , <i>MYO1E</i> , <i>NPHS1</i> , <i>NPHS2</i> , <i>PLCE1</i> , <i>PTPRO</i> , <i>SCARB2</i> , <i>SMARCAL1</i> , <i>TRPC6</i> , <i>WT1</i>	- <i>CFH</i> , <i>CFI</i> , <i>CFB</i> , <i>MCP</i> , <i>C3</i> - <i>CFHR1</i> , <i>CFHR3</i> , <i>CFHR5</i>

PLA2R, M-type phospholipase A2 receptor; Anti-AR Ab, anti-aldose reductase antibody; Anti-SOD2, anti-manganese superoxide dismutase; Anti-NEP Ab, anti-neutral endopeptidase; CLC-1, cardiotrophin-like cytokine-1; APFA, alternative pathway functional activity; MAC, membrane attack complex; C3Nef, C3 nephritic factor; SMA, smooth muscle actin; MCP-1, monocyte chemoattractant protein; MBL, mannose-binding lectin; L-FABP, L-fatty acid binding protein; β -NAG, N-acetyl-beta-glucosaminidase; ua1m, a1-microglobulin.