Fibrillary glomerulonephritis and DnaJ homolog subfamily B member 9 (DNAJB9)

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Abstract
Fibrillary glomerulonephritis (FGN) is a rare glomerular disease that is diagnosed based on presence of fibrils in glomeruli. The fibrils are typically non-congophilic, randomly oriented and measure 12-24 nm. Traditionally, electron microscopy (EM) has been an important tool to aid in diagnosis of FGN by identifying the fibrils and to distinguish it from other entities that could mimic FGN. However, recently DnaJ homolog subfamily B member 9 (DNAJB9) has emerged as both a specific and sensitive biomarker in patients with FGN. It allows prompt diagnosis and alleviates reliance on EM. DNAJB9, is a co-chaperone of heat shock proteins (HSPs) 70, and is involved in endoplasmic reticulum (ER) protein folding pathways. But its role in the pathogenesis of FGN remains elusive. DNAJB9 may act as a putative antigen or alternatively it may secondarily bind to misfolded IgG in the glomeruli. These hypotheses need future studies to elucidate the role of DNAJB9 in pathogenesis of FGN. Treatment regimen for FGN has been limited due to paucity of studies. Most patients receive combination immunosuppressive regimens. Rituximab has been studied the most in FGN and it may delay disease progression. Prognosis of FGN remains poor and 50% require dialysis within 2 years of diagnosis. Despite its poor prognosis in native kidneys, the rate of recurrence post transplantation is low (20%) and patients as well as allograft outcomes are similar to non-FGN patients.
Introduction
Fibrillary glomerulonephritis (FGN) is a rare glomerular disease that was first reported in a patient with nephrotic syndrome in 1977, however, it was not recognized as a distinct entity until 1983. Traditionally, FGN is diagnosed by the presence of non-congophilic, haphazardly oriented, 12-24 nm diameter fibrils in glomeruli. Therefore, until recently, electron microscopy (EM) was considered essential in making the diagnosis of FGN in order to identify the size of the fibrils and their orientation. However, sole reliance on EM to make the diagnosis of FGN posed several diagnostic challenges including a subtype of FGN that had congophilic fibrils, in addition to the overlapping size of the fibrils with other diseases that could mimic FGN. Misinterpretation of the EM could result in making an incorrect diagnosis and recommending inappropriate management. The discovery of DnaJ homolog subfamily B member 9 (DNAJB9) in 2017 has led to a paradigm shift in diagnosing FGN. With an impressive sensitivity and specificity, DNAJB9 has become the new gold standard for diagnosis of FGN and has replaced the overreliance on EM. This review will focus on the clinico-pathological aspect of FGN with emphasis on characteristics, utility and potential mechanistic pathway by which DNAJB9 may play a role in the pathogenesis of FGN.

Epidemiology
As a rare entity, there are no population based studies reporting on the incidence rate of FGN in general population. However, several cross-sectional studies have reported on the prevalence of FGN on renal biopsy which ranges between 0.12 to 0.6%. FGN tends to affect Whites in their fifth and sixth decades of life. Older series suggest disease is more common in females but two recent European series report a slightly higher male predominance (Table 1). Although the exact pathophysiology of FGN remains elusive, there are several conditions that have been associated with FGN. Solid and hematologic malignancies are common in patients with FGN. The prevalence of malignancy in these cohorts have ranged between 4 to 23%. No causal relationship has been established thus far between malignancy and FGN. It is possible that there are coexisting risk factors (such as similar age groups) that increase both the risk of malignancy and FGN. Infections, particularly viral infections, are reported in several case series of FGN. Hepatitis C virus (HCV) is the most common viral infection associated with FGN (ranging between 3 to 27%) and more commonly seen in African Americans. Hepatitis B, on the other hand, is much less common. Human immunodeficiency virus (HIV) infection is also linked to FGN but most cases are with concurrent HCV infection. Non-viral infection-associated FGN is extremely rare and there is only a single case report of a patient who developed FGN in the setting of bone tuberculosis. Autoimmune diseases represent 10-30% of FGN patients which includes but is not limited to autoimmune thyroid disorders, rheumatoid arthritis, Sjogren’s syndrome, systemic lupus erythematosus and inflammatory bowel disease.

Monoclonal gammopathy and FGN
Fibrillary GN has been associated with monoclonal proteins. A pathologic diagnosis of monotypic FGN is established when the IgG deposits exhibit both light chain isotype and IgG subtype restriction. Presence of monoclonal protein in the blood or urine of a patient with FGN is not enough to make a diagnosis of monotypic-FGN if the isotype of IgG does not match the
deposits on the kidney biopsy and should not be called monotypic-FGN. It rather represents the coincidental finding of monoclonal protein in a patient who happens to have FGN. Monotypic-FGN is indeed quite rare.6,8,10,11,13 The rate of monoclonal-FGN tends to be higher in congophilic FGN than in non-congophilic FGN.4 It is now suggested to use paraffin immunofluorescence (IF) in monotypic FGN identified by routine IF in order to make the diagnosis of light chain-restricted monotypic FGN. Paraffin IF has been shown to unmask polytypic deposits in over half of the patients who were thought to have monotypic-FGN on regular IF.19 When confirmed by paraffin IF and IgG subclass staining, DNAJB9-positive monotypic-FGN accounts for less than 1% of FGN cases.20

Clinical manifestation
Patients with FGN frequently present with nephritic and/or nephrotic syndrome. Urine microscopy typically shows hematuria (50-90%) and proteinuria.21 Over 90% of FGN patients have proteinuria of at least 1 g/day and approximately 40-50% of FGN patients presents with nephrotic syndrome.8,10 More than half the patients are hypertensive.8,10 Average serum creatinine upon presentation is over 2 mg/dL with estimated glomerular filtration rate (eGFR) below 30 ml/min/1.73m².10 In rare instances, other glomerular diseases may simultaneously be present with FGN. These patients tend to have rapidly progressive glomerulonephritis as their initial presentation.22-25 Even though systemic symptoms is not part of the clinical manifestation of FGN, extra-renal fibrillary deposits have been reported in several other organs such as pancreas, spleen, lungs, and liver.26 Complement level are typically normal and only small proportion of patients (<10%) have low complement levels on presentation. These patients typically have a co-existing autoimmune disease.3,11,13

Pathological studies

Light microscopy
Renal pathology in FGN is myriad and occasionally can mimic other glomerular pathology. The patterns of injury include mesangioproliferative (MesGN), membranoproliferative (MPGN), endocapillary proliferative, crescentic/necrotizing, membranous, and diffuse sclerosing GN. MesGN is one of the more common patterns of injury and is associated with the most favorable prognosis (Figure 1). On the other hand, diffuse sclerosing GN is the least common and portends the poorest prognosis among FGN patients.8 The earliest phase of FGN is characterized by mesangial expansion and hypercellularity. As the disease progresses from a MesGN pattern of injury to an endocapillary proliferative or MPGN pattern of injury there is thickening of the capillary walls with global or segmental basement membrane double contouring. Endocapillary proliferation may be seen with capillary luminal occlusion with infiltrating inflammatory cells. Global or segmental basement membrane thickening and spike formation is noted in the less common membranous pattern. Focal cellular or fibrous crescents may present in up to 50% of patients but diffuse crescentic lesion is rare (about 5%).10,21 Deposits in FGN are typically non-argyrophilic and are variably periodic acid-Schiff positive.21 Traditionally, FGN deposit was considered to be non-congophilic, however, cases of congophilic FGN have been reported.4 These cases are confirmed by proteomics study to be FGN.

Immunofluorescence microscopy
Immunofluorescence typically shows smudgy staining with polyclonal IgG and C3 in mesangial and glomerular capillary walls. Staining for IgA, IgM and C1q are occasionally noted but have lower intensity than IgG (Figure 1). IgG4 is the most common subtype of IgG present in the FGN deposits. Pseudo-linear pattern mimicking anti-GBM disease may be observed. The two conditions may co-exist, but this is uncommon. As discussed above, monotypic deposits are rare and if present, one should proceed with IF on pronase-digested paraffin embedded tissue to discern monoclonality.

Electron microscopy
Traditionally EM was considered to be the gold standard for FGN diagnosis prior to the era of DNAJB9. FGN fibrils are haphazardly oriented, and straight similar to amyloid fibrils. Fibril size in FGN typically range between 12-24 nm and are larger than amyloid fibrils (8-15 nm). The deposits in the earliest stage of the disease are noted within the mesangium. They are associated with increased mesangial matrix. The deposits are also noted along the glomerular basement membranes in a similar haphazard arrangement. There is associated podocyte injury with foot process effacement. Extra-glomerular fibrillary deposits can rarely be seen in the tubular basement membrane, peritubular capillaries, arterioles and interstitium. The morphology and size of fibrils may lead to diagnostic conundrum particularly in the setting of overlapping fibril size and limited sample for EM (Figure 2). Table 2 summarizes diseases that may mimic FGN and ways to differentiate them.

DNAJB9 in FGN
What is DNAJB9?
DNAJB9 was first identified in bacteria as a cofactor of DnaK. It was categorized into 3 subtypes based on the degree of conservatory domain with the E.coli DnaJ. The J domain of DnaJ poses His-Pro-Asp (HPD) motif which stimulates hydrolysis of ATP and is of importance in interaction with heat shock protein (HSP) 70 by stimulating hydrolysis of ATP. DNAJB9 homologues are ubiquitous and are present in several organelles, cell types and organisms. DNAJB9, one of the mammalian ER-localized DnaJ type II homologue to be discovered, has 223 amino acids with molecular weight of 26 kDa. It is also highly conserved. Human DNAJB9 shares approximately 91% similarity with mice DNAJB9. Even though DNAJB9 (similar to other DnaJ homologue) are ubiquitous, they are found preferentially in organs with well-developed ER such as liver, placenta and kidneys. DNAJB9 acts as a co-chaperone of HSP70 which its role involves folding and assembly of nascent proteins, and sensing ER stress. DNAJB9 and HSP70 are upregulated in the setting of ER stress which leads to activation of ER-associated degradation (ERAD) and unfolded protein response (UPR) in order to mitigate misfolded protein. DNAJB9 also promotes protein refolding after stress subsides. ERAD plays an important role in proteasomal degradation in the ER for ER-resident protein. UPR, one of the key protein homeostasis pathways, is activated during ER stress. Its downstream effects are myriad ranging from reduction of influx of newly synthesized protein in to the ER, increase clearance of misfolded protein, improved protein folding, activation of amino acid metabolism, improvement of antioxidant response, and cellular apoptosis depending on the degree of ER
stress. In non-ER stressed state, DNAJB9 is a selective repressor of inositol-requiring enzyme 1 (IRE1) (one of the main sensors of UPR). It binds to luminal domain of IRE1 and hydrolyzes immunoglobulin binding protein (BiP)-ATP by its J-domain. Subsequently, BiP-ADP will bind to IRE1 and disrupt IRE1 dimerization which in turn suppresses UPR. On the other hand, increased unfolded protein in ER stressed state will compete for DNAJB9 and BiP, therefore IRE1 dimerization occurs leading to UPR. This mechanism may actually be beneficial and prevent cell death from ER stress (Figure 1).

Given the role of DNAJB9 in protein quality control, it has been studied in several conditions including B-cell maturation and antibody production, surfactant protein production in interstitial lung disease, chronic ER stress and UPR activation in hepatitis C infection, metabolic pathways, and angiogenesis. Furthermore, upregulation of DNAJB9 could enhance hematopoietic stem cells ability to withstand ER stress resulting in faster repopulation. These studies have emphasized the importance of DNAJB9 in cell maturation, metabolism and functions in multitude of disease processes.

**Discovery of DNAJB9 in FGN**

Earlier application of mass spectrometry for protein characterization was mainly performed in amyloidosis. It has, since, transformed the diagnostic paradigm of amyloidosis and has become the gold standard for amyloidosis diagnosis. This technique since has been studied in other glomerular diseases that have glomerular deposits, which led to the discovery of DNAJB9. DNAJB9 was found to be overabundant in FGN glomeruli through the use of laser microdissection-assisted liquid chromatography-tandem mass spectrometry (LC-MS/MS) and was reported by our group from Mayo Clinic and the University of Washington in 2017 at the same time. In the study from the Mayo Clinic group, there were 24 FGN patients, 145 amyloid patients, 72 patients with glomerular diseases other than FGN (NFGNGD) and 12 normal controls. They discovered several overabundant proteins in FGN glomeruli including complement factors and DNAJB9. Even though, DNAJB9 was the fourth most abundant protein in FGN glomeruli, it was the most specific. DNAJB9 was not detected in normal controls or those with NFGNGD. This distinction made DNAJB9 a strong biomarker for FGN. Another important proteomic finding was the overabundance of Ig-γ, Ig-κ, and Ig-λ which is consistent with known features of FGN. Dual IF staining of DNAJB9 and IgG demonstrated the co-localization of DNAJB9 and IgG with typical pattern of IgG deposition in FGN. With IgG co-localization, it was implied that DNAJB9 was indeed overexpressed extracellularly.

Following the discovery of DNAJB9, immunohistochemistry (IHC) testing for DNAJB9 was developed and was tested in a large group of patients (84 with FGN, 21 with amyloidosis, 98 with NFGNGD, and 11 healthy subjects). The stain demonstrated strong, homogenous smudgy DNAJB9 staining in the glomerular deposit in all FGN patients except 2 patients who stained negative for DNAJB9. These 2 cases both had negative staining for light chain but were positive for heavy chain. Both patients had evidence of heavy chain monoclonal gammopathy. One of the patients was subsequently found to have a truncated monoclonal gamma 1 heavy chain secondary to a complete variable region (VH) deletion. In the non-FGN group, only a single patient had positive staining for DNAJB9. In this particular case, the staining was very focal. In
addition, the patient was noted to be a smoker and had hepatitis C infection. The authors believe that this patient was perhaps having an early stage of FGN and as a result the sample glomeruli for EM did not contain fibrillary deposition.6 IHC staining in a similar study but a smaller group of patients showed the same results.5 IHC staining in 11 FGN patients yielded 100% positive rate but was negative in all 21 non-FGN controls.5 There were no false negative or false positive cases in this study likely due to the smaller size of their cohort.5 Therefore, IHC for DNAJB9 has excellent sensitivity (97.6%) and specificity (99.2%) in FGN.6

Postulated pathophysiology of DNAJB9 in FGN
The pathogenesis of FGN has remained elusive. Since DNAJB9 is a strong biomarker for FGN, it is likely that DNAJB9 plays a role in the pathophysiology of FGN. It has long been postulated that FGN is an immune-complex disease and IgG deposits (primarily IgG4) are what is polymerized into fibrils.9,21 However, it has never been proven that the fibrils are composed of IgG and thus the possibility of in-situ binding of IgG to previously formed fibrils has been raised.52 Based on this, two potential pathogenic mechanisms of DNAJB9 in FGN have been proposed.21 The first theory hypothesizes that DNAJB9 itself is the putative antigen in FGN which gets deposited in the kidney and forms fibrils. This is then followed by formation of autoantibodies (primarily IgG4) to the DNAJB9 (Figure 3).6,18 This hypothesis is supported by the fact that DNAJB9 is overabundant in FGN glomeruli,5,50 and is detected first in the cases of recurrent FGN post kidney transplantation (despite the absence of IgG staining on IF).53 This suggests that DNAJB9 deposits precede the IgG deposition suggesting that the most likely initial step in the pathogenesis of the disease is the deposition of DNAJB9 in the kidney.53 However, so far no auto-antibodies against DNAJB9 have been identified.21 In addition, the DNAJB9 protein that is found in FGN is intact without evidence of truncation and thus far no genetic alteration in DNAJB9 gene has been identified among FGN patients.50 Therefore the reason for the upregulation of DNAJB9 in patients with FGN remains unclear.50 The source for overproduction of DNAJB is also unclear. It is unlikely that the source is the kidney itself, as the disease can recur post transplantation.7,53,54 In addition, there is no evidence of enrichment of other components of ER stress in the glomeruli.5 Moreover, DNAJB9 is not transcriptionally upregulated in the kidneys of FGN patients.55 It is more likely that DNAJ9 is overproduced extra-renal and is then deposited in the glomeruli which subsequently incites inflammatory response. Supporting the extra-renal source for DNAJB9 are also the reports of fibril deposits outside the kidney.9

The other proposed mechanism for the role of DNAJB9 in pathogenesis of FGN is that DNJAB9 itself is not pathogenic but rather it secondarily bind to the misfolded IgG proteins. DNAJB9 is pivotal in the UPR and ER protein quality check.33,40,56 It is conceivable that in patients with FGN, the underlying pathology is an increase in the formation of misfolded IgG (for example due to increase in ER stress) and DNAJB9 subsequently binds to the misfolded IgG. This may potentially explain the increased rate of FGN in patients with underlying autoimmune diseases, malignancy, and infections. Indeed hepatitis C has been shown to cause chronic ER stress.43 The ability of DNAJB9 to bind to misfolded protein is supported by a recent study in mice which found that DNAJB9 specifically recognized aggregation-prone regions in misfolded immunoglobulin (which is normally buried in the normal folding process) and DNAJB9 was
upregulated in order to bind to the misfolded immunoglobulin.\textsuperscript{57} It is therefore possible that in patients with FGN the misfolded IgG is deposited in the kidney first and then the DNAJB9 secondarily binds to this misfolded IgG in the kidney (Figure 4). It has also been proposed that DNAJB9 is already bound to the misfolded IgG as it is secreted from plasma cells and is then deposited in the kidney (Figure 4).\textsuperscript{21} The missing gap with this theory is that once the DNAJB9 is bound to the misfolded protein, it should enter ERAD process to prevent cellular toxicity,\textsuperscript{42} and how the complex escapes this step remains unknown. In addition, the finding of DNAJB9 preceding IgG deposition in recurrent FGN post kidney transplantation argues against DNAJB9 secondarily binding to IgG as one would have expected to see IgG deposits either first or simultaneously with DNAJB9 deposition.\textsuperscript{53}

\textit{Serum study of DNAJB9}

Serum DNAJB9 level has been studied in FGN in light of the discovery of DNAJB9 in kidney biopsies. Serum levels of DNAJB9 can be measured by immunoprecipitation-linked multiple reaction monitor assay.\textsuperscript{58} FGN patients had significantly higher serum levels of DNAJB9 (median serum DNAJB9 1.03 nM) than in normal population (median 0.23 nM), multiple myeloma (median 0.29 nM), NFGNGD (median 0.32 nM) and AL amyloidosis (0.43 nM) patients.\textsuperscript{58} Serum levels of DNAJB9 was inversely associated with eGFR but this association was not seen in other control groups (except in AL amyloidosis).\textsuperscript{58} Therefore, it is possible that both overproduction and decreased excretion of DNAJB9 result in higher serum DNAJB9 levels in FGN patients.\textsuperscript{58} Serum DNAJB9 levels can predict the odds of finding FGN. For every 1nM increase in serum DNAJB9 levels, the odds of having FGN increases 54.37 times after adjusting for eGFR.\textsuperscript{58} Thus far, serum DNAJB9 measurements have been only done at a single center and therefore independent validation of this method and the results by other investigators is needed. In addition, the technique used in this study is technically complex and costly which would limit its applicability to clinical practice. Other techniques such as enzyme-linked immunosorbent (ELISA) that are quicker and less technically challenging would be preferable. However, future studies are needed to validate other methods.\textsuperscript{58}

Thus far, there is only a single study that has evaluated the change in DNAJB9 levels over time after therapy. In this 12-month prospective pilot trial of rituximab in FGN, there was no change in DNAJB9 levels overtime after treatment with rituximab. In addition, there was no association between change in DNAJB9 levels and eGFR overtime.\textsuperscript{59} It is difficult to draw any definite conclusions from this study as the sample size was limited (n=11) and only 3 patients achieved partial remission at the end of the study.\textsuperscript{59} Ideally, anti-DNAJB9 (equivalent to PLA2R antibody in membranous nephropathy) would be a useful marker for diagnosing and monitoring the response to therapy. However, this autoantibody is yet-to-be discovered.

\textbf{Treatment}

Outcomes of patients with FGN remains poor. Approximately 50% of patients reach end-stage kidney disease (ESKD),\textsuperscript{8,10} with median time to ESKD ranging between 24 - 44 months.\textsuperscript{8,11} Patients with older age, male sex, and higher proteinuria, and lower eGFR at diagnosis are more likely to progress to ESKD.\textsuperscript{10,18} In the absence of any therapy, close to 80% of patients reach ESKD. Rarely, few patients with FGN may enter spontaneous remission with conservative
Currently, there are no standard regimens for treatment of patients with FGN and there have been no randomized controlled trials to help guide therapy. Available data are primarily generated from retrospective case series and case reports. Moreover, in the absence of an understanding of the underlying pathophysiology of the disease, there are no targeted therapies available. Most patients receive anti-proteinuric agents with angiotensin converting enzyme inhibitor (ACE-I) or angiotensin receptor blockade (ARB). Other anti-proteinuric measures including strict blood pressure control, low salt diets, weight loss and dietary protein restriction particularly in those with nephrotic range proteinuria are recommended.

Even though immunosuppression is usually initiated in most patients, there is no clear evidence that immunosuppression results in improvement of renal outcomes. Steroids alone were historically used with varying degrees of success. Cyclophosphamide, cyclosporine, mycophenolate mofetil, azathioprine, lenalidomide, rapamycin have also been used without any significant benefit. Rituximab is thus far the most promising agent that has been studied in FGN. Early case reports demonstrated that Rituximab could stabilize kidney function and significantly reduced proteinuria. A small retrospective study showed that a third of the patients (4 out of 12) had stable kidney function over the follow-up period (defined as stable or increase in creatinine < 25%). Similarly, the largest multi-institutional cohort of FGN showed that of all the immunosuppressive agents used, rituximab was the only treatment that was associated with stable kidney function and reduction of ESKD. Thus far there has only been one prospective study evaluating the efficacy of rituximab in patients with FGN. In this 12-months pilot study, 11 FGN patients were treated with 2 courses of rituximab over a span of 6 months and were followed for 1 year. In this study, rituximab was associated with stabilization of renal function and proteinuria decreased from 4.4 to 1.9 g/24hr (though the reduction in proteinuria did not reach statistical significance, p=0.06). In this study, 3 out of 11 patients achieved partial remission (defined as having < 10% decline in creatinine clearance and proteinuria < 3g/24hr with at least 50% reduction from baseline). One patient that had achieved partial remission entered complete remission 22 months after trial completion. It is possible that with larger sample size and longer follow-up time, we may be able to capture the benefits of rituximab in FGN. Table 3 summarizes previous studies of treatment in FGN.

**Recurrence post kidney transplantation**

There is paucity of studies evaluating transplant outcomes in FGN patients. The rate of recurrence in allograft varies between studies due to heterogeneity of diagnostic criteria and the difficulty of distinguishing between FGN and its mimickers prior to the DNAJB9 discovery. Studies in pre-DNAJB9 era showed that the rate of recurrence ranged between 8-50%. However, our recent study using DNAJB9 as a diagnostic tool demonstrated that the rate of recurrence in allograft is 21%. FGN tends to recur late and most patients have recurrence after 5 years. The earliest recurrence was reported at 2 years post-transplant. Despite its poor prognosis in native kidney, the recurrent FGN in allografts do not appear to significantly affect allograft or patient outcomes. Indeed, the recurrent FGN typically presents insidiously and some of the patients were diagnosed based on protocol renal biopsies (without overt clinical manifestations). In general, post kidney transplantation, FGN patients have comparable
outcome to other ESKD patients with 100% and 67% of patient and renal-allograft survival at 10 years, respectively.\textsuperscript{7} Data regarding treatment of recurrent FGN in renal allograft remains limited. The Mayo cohort reports no adjustment in immunosuppression regimen in all recurrent cases. One patient lost the allograft and underwent a second preemptive kidney transplantation. The other 2 patients have stable kidney functions at 10 and 13 years post-transplant respectively.\textsuperscript{53}

**Future Directions and Conclusion**
DNAJB9 is an excellent biomarker for FGN and has become the new gold standard for FGN diagnosis. Immunohistochemistry stain of DNAJB9 has now replaced the EM and allows for prompt diagnosis of FGN. Despite its strength, the role of DNAJB9 in FGN pathogenesis remains elusive. There are currently two hypothesis regarding the role of DNAJB9 in pathogenesis of FGN which include DNAJB9 as an autoantigen versus misfolded immunoglobulin inducing DNAJB9 upregulation and DNAJB9 binding secondarily to the misfolded protein. These hypothesis highlight the gaps in our understanding of the disease pathophysiology and future studies are required to better understand the role of DNAJB9 in pathogenesis of FGN. We propose that future FGN clinical trials incorporate DNAJB9 in making the diagnosis of FGN (requiring IHC staining) and also recommend measuring serum DNAJB9 levels in order to garner a better understanding of longitudinal changes of DNAJB9 in the course of the disease. Urine DNAJB9 deserves more attention and may serve as a potential useful non-invasive biomarker in the future.

Since the first case of FGN four decades ago, our understanding of FGN has evolved substantially. Yet, FGN patients face grim prognosis owing to lack of effective therapy. This emphasizes the needs for future clinical trials with robust design, large sample size and longer follow-up period in order to capture significant changes. Furthermore, as a rare entity, multi-institutional collaboration is required to effectively tackle this disease and provide comprehensive care to our patients.

**Disclosures**
All authors have nothing to disclose.

**Author Contributions**
N Klomjit: Conceptualization; Data curation; Resources; Writing - original draft; Writing - review and editing
M Alexander: Data curation; Resources; Supervision; Writing - review and editing
L Zand: Conceptualization; Data curation; Resources; Supervision; Visualization; Writing - review and editing
Reference


## Table 1: Clinical presentation of FGN patients from the most recent case series

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Nasr et. al (2011)\textsuperscript{10}</th>
<th>Javaugue et. al (2013)\textsuperscript{11}</th>
<th>Payan Schober et. al (2017)\textsuperscript{13}</th>
<th>Nasr et. al (2017)\textsuperscript{6}</th>
<th>Andeen et. al (2019)\textsuperscript{18}</th>
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<tbody>
<tr>
<td>Sample size (n)</td>
<td>66</td>
<td>27</td>
<td>42</td>
<td>84</td>
<td>266</td>
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<tr>
<td>Age (years)</td>
<td>53 ± 12</td>
<td>59 (25-82)</td>
<td>59 (25-82)</td>
<td>59 (21-80)</td>
<td>61 (24-87)</td>
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<tr>
<td>Female (%)</td>
<td>36 (55%)</td>
<td>11 (41%)</td>
<td>25 (60%)</td>
<td>74 (62%)</td>
<td>173 (65%)</td>
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<td>White (%)</td>
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<td>30 (71%)</td>
<td>NA</td>
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<td>Diabetes (%)</td>
<td>13 (20%)</td>
<td>6 (22%)</td>
<td>28 (70%)</td>
<td>20 (24%)</td>
<td>60 (22%)</td>
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<td>Hypertension (%)</td>
<td>47 (71%)</td>
<td>19 (70%)</td>
<td>NA</td>
<td>52 (62%)</td>
<td>144 (54%)</td>
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<td>Associated conditions</td>
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<td>Hepatitis C infection (%)</td>
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<td>2 (7%)</td>
<td>7 (27%)</td>
<td>6 (7%)</td>
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<td>Autoimmune diseases (%)</td>
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<td>8 (30%)</td>
<td>4 (10%)</td>
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<td>Hematologic malignancy (%)</td>
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<td>0 (0%)</td>
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<td>6 (2%)</td>
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<td>1 (4%)</td>
<td>6 (12%)</td>
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<td>14 (5%)</td>
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<td>Serum creatinine (mg/dL)</td>
<td>2.1 (0.5-8.3)</td>
<td>NA</td>
<td>3.2 ± 4</td>
<td>2.5 (0.4-12.8)</td>
<td>2.1 (1.46-3.45)**</td>
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<td>Hypocomplementemia</td>
<td>1 (2%)</td>
<td>NA</td>
<td>2 (9%)</td>
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<td>Urinary studies</td>
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<tr>
<td>Urine protein at biopsy (g/d)</td>
<td>5.62 (0.2-20.4)</td>
<td>3.2 (0.5-17)</td>
<td>5.7 ± 5</td>
<td>5.1 (0-20)</td>
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<td>Nephrotic syndrome (%)</td>
<td>24 (38%)</td>
<td>11 (41%)</td>
<td>NA</td>
<td>21 (25%)</td>
<td>39 (15%)</td>
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<tr>
<td>Hematuria (%)</td>
<td>33 (52%)</td>
<td>19 (73%)</td>
<td>36 (97%)</td>
<td>76 (90%)</td>
<td>104 (39%)</td>
</tr>
</tbody>
</table>

Abbreviations: NA: not available

Continuous data represented by either mean ± standard deviation or median (range or interquartile range) depending on original reports. Categorical data represented by number (n) and percentage (%).

Percentage represented in categorical data use the number of available test or data as the denominator.

*118 patients had ethnicity information

** The values are interquartile range
<table>
<thead>
<tr>
<th>Diseases</th>
<th>LM</th>
<th>IF</th>
<th>Congo red stain</th>
<th>EM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrillary GN(^{21})</td>
<td>MesGN, MPGN, MGN, DPGN and DSGN</td>
<td>Smudgy appearance of polyclonal IgG and C3</td>
<td>Mostly negative, rarely positive (4%)(^{4})</td>
<td>Fibril size 12-24 nm; Fibrils are randomly oriented, straight</td>
</tr>
<tr>
<td></td>
<td>Variably argyrophilic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amyloidosis(^{64,65})</td>
<td>Mesangial expansion with acellular matrix, PAS negative, non-argyrophilic Deposits can be present in interstitium/vessel walls</td>
<td>Monotypic staining of either kappa or lambda light chain in AL amyloidosis</td>
<td>Positive</td>
<td>Fibril size 7-12 nm; Fibrils are randomly oriented, solid and non-branching</td>
</tr>
<tr>
<td>Immunotactoid glomerulopathy(^{66})</td>
<td>MPGN pattern (most common)</td>
<td>Mesangial and capillary wall IgG positivity 70% of patients show monotypic staining</td>
<td>Negative</td>
<td>Microtubule size 17- 52 nm; Microtubules have parallel arrangements, centrally hollowed</td>
</tr>
<tr>
<td>Fibronectin glomerulopathy(^{67,68})</td>
<td>MPGN pattern Strongly PAS positive, non-argyrophilic</td>
<td>Usually negative but may show nonspecific staining for Ig and C3</td>
<td>Negative</td>
<td>Fibril size 12-16 nm; Extensive mesangial and subendothelial deposits with focal fibrillary substructures</td>
</tr>
<tr>
<td>Diabetic fibrillosis(^{69})</td>
<td>Mesangial matrix expansion and nodularity Strong PAS positive, argyrophilic (FGN deposits are non-argyrophilic)</td>
<td>Linear glomerular capillary wall and tubular basement membrane staining with IgG and albumin Focal segmental mesangial IgM and C3 staining</td>
<td>Negative</td>
<td>Fibril size 10- 20 nm; Fibrils are seen in short bundles in parallel arrays, non-branching</td>
</tr>
</tbody>
</table>


* The fibril sizes indicated for each disease are the typical reported sizes (fibril sizes can be smaller or larger than what is noted).
### Table 3: Selected literatures of treatment regimen in FGN

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sample size</th>
<th>Regimen</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conservative treatment with RAAS blocking agents</strong>&lt;br&gt;Case series 10</td>
<td>16</td>
<td>ACEI/ARB</td>
<td>2 CR, 2 PR, 8 PRD, 4 ESKD</td>
</tr>
<tr>
<td>Case series 11</td>
<td>14</td>
<td>ACEI/ARB</td>
<td>1 CR, 1 PR, 2 PRD, 10 ESKD</td>
</tr>
<tr>
<td><strong>Corticosteroid</strong>&lt;br&gt;Case reports40</td>
<td>3</td>
<td>Prednisone 1 mg/kg/day</td>
<td>3 CR</td>
</tr>
<tr>
<td>Case report76</td>
<td>1</td>
<td>High dose steroid</td>
<td>NR</td>
</tr>
<tr>
<td>Case series8</td>
<td>9</td>
<td>NA</td>
<td>9 NR</td>
</tr>
<tr>
<td>Case series10</td>
<td>8</td>
<td>NA</td>
<td>8 NR</td>
</tr>
<tr>
<td>Case series11</td>
<td>5</td>
<td>NA</td>
<td>5 NR</td>
</tr>
<tr>
<td>Multi-institutional cohort18</td>
<td>24</td>
<td>NA</td>
<td>16 (67%) ESKD at median follow-up of 28 months</td>
</tr>
<tr>
<td><strong>Cyclophosphamide</strong>&lt;br&gt;Case series11</td>
<td>3</td>
<td>NA</td>
<td>1 PR, 1 PRD, 1 ESKD</td>
</tr>
<tr>
<td>Case report71</td>
<td>2</td>
<td>200 mg/d then 100 mg/d x 6 m + steroid then azathioprine 50 mg/d&lt;br&gt;150 mg/d x 2 weeks then 800 mg/m² pulse monthly x 6 m then azathioprine 50 mg/d</td>
<td>2 PR</td>
</tr>
<tr>
<td>Case report72</td>
<td>1</td>
<td>NA</td>
<td>PR</td>
</tr>
<tr>
<td>Case report73</td>
<td>1</td>
<td>100mg/d x 1 year + prednisone 40 mg/d</td>
<td>PR</td>
</tr>
<tr>
<td>Case report25</td>
<td>1</td>
<td>1 mg/kg/d+ prednisone 60 mg/d with tapering regimen</td>
<td>Improved in creatinine but persistent proteinuria</td>
</tr>
<tr>
<td>Multi-institutional cohort18</td>
<td>9</td>
<td>NA</td>
<td>8(89%) ESKD at median follow-up of 24 months</td>
</tr>
<tr>
<td><strong>Cyclosporine</strong>&lt;br&gt;Case series8</td>
<td>3</td>
<td>NA</td>
<td>1 PR, 2 NR</td>
</tr>
<tr>
<td>Case series10</td>
<td>2</td>
<td>NA</td>
<td>2 NR</td>
</tr>
<tr>
<td>Case series11</td>
<td>4</td>
<td>NA</td>
<td>4 NR</td>
</tr>
<tr>
<td><strong>Mycophenolate mofetil</strong>&lt;br&gt;Case report74</td>
<td>1</td>
<td>500 mg BID x 8 months + prednisone 40 mg/d</td>
<td>Improvement in serum Cr but persistent hematuria and proteinuria (patients died due to non-renal cause 8 months later)</td>
</tr>
<tr>
<td>Case report23</td>
<td>1</td>
<td>1g BID + high dose steroid</td>
<td>ESKD</td>
</tr>
<tr>
<td>Case series10</td>
<td>1</td>
<td>NA</td>
<td>PR</td>
</tr>
<tr>
<td><strong>Lenalidomide</strong>&lt;br&gt;Case series10</td>
<td>2</td>
<td>NA</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Rapamycin</strong>&lt;br&gt;Case series10</td>
<td>1</td>
<td>NA</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Bortezomib</strong>&lt;br&gt;Case series11</td>
<td>1</td>
<td>NA</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Rituximab</strong>&lt;br&gt;Case report62</td>
<td>3</td>
<td>RTX 375 mg/m² x 4-8 doses or RTX 1 g IV x 2 doses</td>
<td>PR</td>
</tr>
<tr>
<td>Case series10</td>
<td>3</td>
<td>NA</td>
<td>NR</td>
</tr>
<tr>
<td>Case series63</td>
<td>12</td>
<td>RTX 1 g IV x 2 doses or 375 mg/m² x 4 doses</td>
<td>4 non-progressors, 3 PRD, 5 ESKD</td>
</tr>
<tr>
<td>Case series11</td>
<td>7</td>
<td>RTX 375 mg/m² x 2 - 4 doses</td>
<td>5 PR, 2 PRD</td>
</tr>
<tr>
<td>Multi-institutional cohort18</td>
<td>8</td>
<td>NA</td>
<td>1 ESKD at median follow-up time of 14 m. Rituximab was significantly associated with lack of progression to ESKD (HR 0.435)</td>
</tr>
<tr>
<td>Pilot prospective trial59</td>
<td>11</td>
<td>RTX g IV x 2 doses and another 2 doses at 6 m</td>
<td>3 PR</td>
</tr>
</tbody>
</table>

Figure 1: Renal Histology of fibrillary GN: A) PAS stain which shows mild mesangial expansion without marked proliferation. B) Jones Methenamine Silver stain showing that the mesangial deposits are silver negative. C) Immunofluorescence studies demonstrates smudgy deposits and pseudo-linear staining with IgG. D) Randomly oriented fibrils are noted with a mean thickness of 12nm. E) DNAJB9 immunohistochemical stains are seen in the glomerulus and focally along the tubular basement membranes.
Figure 2: Diseases that can mimic Fibrillary GN: A) The glomerulus shows expansion of the mesangium with PAS weak acellular deposits of amyloid. The adjacent arteriole also shows amyloid deposits. B) Congo red stain shows apple green birefringence when viewed with polarizing light. C) The ultrastructural studies show randomly oriented non-branching fibrils with a mean diameter of 9 nm. The fibrils form perpendicularly organized aggregates. D, E) Electron microscopy image of immunotachtoid glomerulopathy showing parallel arrays of organized micro-tubular structures are noted. The mean thickness of the fibers is 20 nm. F) Diabetic mesangial fibrillosis is characterized by short bundles of organized fibrils.
Figure 3: DNAJB9 in normal physiology: DNAJB9 is a key co-chaperone of heat shock protein 70 (HSP70) in maintaining cellular protein homeostasis. It has 2 domains known as targeting domain and J-domain. Targeting domain is responsible for binding to its target proteins. On the other hand, J-domain facilitates immunoglobulin binding protein (BiP) (a member of HSP70) to tightly bind with inositol-requiring enzyme-1 (IRE-1). In normal physiological state, DNAJB9 is upregulated during hematopoietic stem cells differentiation, macrophage activation, angiogenesis and plasma cell differentiation. In non-ER stress state, DNAJB9 will binds to IRE-1 and the J-domain will hydrolyze the ATP that binds to BiP. Consequently, BiP-ADP will bind to IRE-1 which prevents IRE-1 dimerization. This in turn will suppress unfolded protein responses (UPR) in the normal state. In ER-stressed state, DNAJB9 together with BiP will bind to the accumulated misfolded proteins and lead the misfolded protein to endoplasmic reticulum associated protein degradation (ERAD). On the other hand, since DNAJB9 and BiP are less available, IRE-1 could dimerize and activate UPR leading to another pathway of protein degradation.
Figure 4

a

Non-truncated but misfolded DNAJB9

Cells undergo ER stress and release unstable/misfolded DNAJB9

Incite immune response

Plasma cell

Secrete autoantibody (not yet identified)

Fibrillogenesis in kidneys and other organs

b

Non-truncated but misfolded DNAJB9

Plasma cells secrete misfolded IgG

Or

Plasma cells secrete misfolded IgG with DNAJB9 complex

Fibrillogenesis in kidneys and other organs
Figure 4: Postulated pathophysiology of DNAJB9 in FGN: There are two main postulated pathophysiology of DNJB9 in FGN (a) Upon ER stress, misfolded non-truncated-DNAJB9 is upregulated and secreted into systemic circulation. Misfolded DNAJB9 will deposit in the kidneys and at the same time will stimulate immune response leading antibody production which subsequently binds to the deposited DNAJB9 in the kidneys culminating in fibrillogensis. (b) Plasma cells secrete misfolded IgG which is then deposited in glomerular mesangium. Other cells that undergo ER stress secrete DNAJB9 which then binds to previously deposited IgG in the mesangium forming FGN fibrils (top panel). Alternatively, plasma cells could undergo ER stress and secrete misfolded IgG with DNAJB9 complex. This complex will get deposited in the mesangium resulting in the fibrillogenesis (bottom panel).