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Diagnostic Approach to Glomerulonephritis With Fibrillar IgG Deposits and Light Chain Restriction

Satoru Kudose[1], Pietro Canetta[2], Nicole K. Andeen[3], M. Barry Stokes[1], Ibrahim Batal[1], Glen S. Markowitz[1], Vivette D. D'Agati[1] and Dominick Santoriello[1]

[1]Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, New York, USA; [2]Department of Medicine, Division of Nephrology, Columbia University Irving Medical Center, New York, New York, USA; and [3]Department of Pathology, Oregon Health & Science University, Portland, Oregon, USA

Introduction: The pathologic approach to glomerulonephritis (GN) with fibrillar IgG deposits and light chain restriction remains a diagnostic challenge.

Method: All GN with fibrillar deposits of IgG and apparent light chain restriction on standard immunofluorescence on frozen tissue (IF-F) accessioned at the Columbia Renal Pathology Laboratory from 2012 to 2019 were identified. Additional studies including staining for Congo red, DNAJB9, IgG subtypes, and immunofluorescence on pronase-digested paraffin sections (IF-P) were performed.

Result: Based on the results, biopsy samples were reclassified as polytypic DNAJB9-positive fibrillary glomerulonephritis (pFGN, n = 14), monotypic DNAJB9-positive FGN (mFGN, n = 7), GN with polytypic DNAJB9-negative fibrillar IgG deposits (n = 2), and GN with monotypic DNAJB9-negative fibrillar IgG deposits (n = 6). Among DNAJB9-positive FGN samples, IgG subtype staining was able to exclude monotypic deposits by demonstrating reactivity for ≥2 IgG subtypes (usually IgG1 and IgG4) in 67% (14 of 21), including 9 that would have been misclassified as monotypic by IF-F and IF-P alone. Monotypic DNAJB9-positive fibrillary glomerulonephritis (FGN) was not associated with monoclonal gammopathy in 5 of 6 patients. GN with monotypic DNAJB9-negative fibrillar IgG deposits exhibited focal parallel fibril alignment and frequent association with chronic lymphocytic leukemia, but lacked the diagnostic microtubules of immunotactoid GN.

Conclusion: A systematic diagnostic approach with ancillary techniques is essential for proper classification and assignment of monoclonal gammopathy of renal significance status in cases of GN with fibrillary IgG deposits and light chain restriction by IF-F.

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The diagnostic approach to fibrillary glomerulonephritis (FGN) has evolved considerably since its initial description in 1977.[1] Historically, fibrillary glomerulonephritis was morphologically defined by the presence of fibrils with the following: (i) random orientation, (ii) no hollow cores at magnifications greater than ×30,000, (iii) width of <30 nm, and (iv) positive staining for immunoglobulins by immunofluorescence.[2–4] However, with the recent discovery of DnaJ homolog subfamily B member 9 (DNAJB9) as a specific constituent of the glomerular deposits in FGN,[5,6] Fibrillary glomerulonephritis is increasingly being defined by the presence of DNAJB9 deposition.

Importantly, a rare subset of FGN demonstrates apparent light chain restriction by routine immunofluorescence on frozen sections (IF-F). This variant is sometimes referred to as "monoclonal" FGN,[3,7] and its status as a form of monoclonal gammopathy of renal significance (MGRS) is currently controversial.[8] According to the 2 recent studies, prevalence of hematolymphoid neoplasm among specimens classified as "monoclonal" FGN was similar to those without light chain restriction, casting doubt on the status of "monoclonal" FGN as a form of MGRS.[7,9]

Despite the important therapeutic implications of this issue, most prior studies lack DNAJB9

Correspondence: Dominick Santoriello, Department of Pathology, Columbia University Irving Medical Center, 630 West 168th Street, Room VC14-224, New York, New York 10032, USA. E-mail: ds3356@cumc.columbia.edu

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immunostaining, pronase immunofluorescence (IF-P), and IgG subclass staining,[3,7,10] and do not use a systematic approach to differentiate "monoclonal" FGN from its mimickers, such as immunotactoid glomerulonephritis, glomerulonephritis with organized microtubular monoclonal deposits,[2–4,11] or DNAJB9-negative variant of FGN.[10] Because patients with MGRS may receive extensive workup or treatment for possible hematolymphoid neoplasm,[8] the importance of a systematic diagnostic approach to precisely characterize these entities cannot be overemphasized. Herein, we systematically evaluate cases of glomerulonephritis with apparent light chain restriction and fibrillar deposits using Congo red stain, DNAJB9 immunostain, immunofluorescence for IgG subtypes, and IF-P to determine their usefulness in defining the disease spectrum, distinct disease subsets, and their relative associations with dysproteinemic conditions.

MATERIALS AND METHODS

All native kidney biopsy samples accessioned at the Columbia Renal Pathology Laboratory from 1 January 2012 to 30 August 2019 with the terms "fibril," "fibrillary," or "fibrillary" in any field were retrieved and re-reviewed by 4 renal pathologists (SK, DS, VD, and GSM). All biopsies with IgG-dominant deposits exhibiting predominantly fibrillar substructure and apparent light chain restriction were included in the study. Light chain restriction was defined as the presence of 1 light chain only or the presence of staining for 1 light chain with ≥2+ intensity and at most trace staining for the other light chain (scale of 0−3+) on routine frozen immunofluorescence. Biopsy samples diagnosed as lupus nephritis with organized deposits, proliferative glomerulonephritis with monoclonal IgG deposits exhibiting focal organized substructure, and immunotactoid glomerulonephritis (defined by the presence of microtubular deposits with visible hollow cores at ×50,000 or lower magnification) were excluded from the study. Biopsies without available tissue for DNAJB9 immunostain and/or IgG subclass staining were also excluded from the study.

All kidney biopsy samples were processed according to standard techniques for light microscopy (LM), immunofluorescence (IF), and electron microscopy (EM) and were interpreted by 1 of 6 pathologists. For immunofluorescence, 3-μm frozen sections (IF-F) were stained with fluorescein isothiocyanate (FITC)−conjugated rabbit anti-human IgG, IgM, IgA, C3, C1q, kappa (κ) and lambda (λ) light chain (Dako, Carpinteria, CA). For all cases with available remaining frozen tissue, IgG subtypes were determined using polyclonal FITC-conjugated antibodies to IgG1-4 (The Binding Site, Birmingham, UK). For all cases with remaining formalin-fixed and paraffin-embedded (FFPE) tissues, Congo red stain, immunohistochemical stain for DNAJB9 (Sigma-Aldrich, St. Louis, MO), and immunofluorescence for IgG, κ, and λ was performed on pronase-digested FFPE tissue (IF-P) as previously published.[12] The diameters of the fibrils were re-measured independently by 4 renal pathologists (SK, DS, VD, and GSM), and the mean diameter and range of combined measurements were determined. Repeat electron microscopic examination at up to ×100,000× was performed in all cases with DNAJB9-negative deposits to exclude the presence of hollow cores.

The submitting physicians provided the following information: patient demographics, past medical history including hypertension, diabetes and autoimmune disease, serum creatinine at presentation, urine protein-to-creatinine ratio or 24-hour urine protein level, dipstick proteinuria (on a scale of 0 or trace, 1−4+), serum albumin, presence of hematuria (>5 cells/hpf), edema, anti-nuclear antibody, serum complements, hepatitis B surface antigen, hepatitis C antibody, serum-free κ-to-λ light chain ratio, serum and urine electrophoresis, and bone marrow biopsy, if available. In addition, follow-up information regarding serum creatinine, urine protein-to-creatinine ratio or 24-hour urine protein level, dipstick proteinuria (on a scale of 0 or trace, 1−4+), serum albumin, type of treatment, and presence of hematolymphoid neoplasm at the time of most recent follow-up were obtained.

The following pathologic parameters were collected: predominant light microscopic pattern, number of total and globally sclerotic glomeruli, percentage of cortical tubulointerstitial scarring, vascular sclerosis (on a scale of none, mild, moderate, and severe), presence of coexisting disease process, immunofluorescence intensity for all immunoreactants (on a scale of 0, trace, 1, 2, or 3+), presence of electron-dense deposits and their location, mean and range for fibril diameters, presence of hollow cores, coexistence of amorphous deposits, and percentage of foot process effacement.

This study was approved by the Institutional Review Board of Columbia University Irving Medical Center.

RESULTS

We identified 36 biopsy samples from 35 patients showing glomerulonephritis with fibrillar deposits of IgG and light chain restriction on routine frozen immunofluorescence (IF-F). Of these, 7 were excluded from further analysis because of lack of available tissue for IgG subtype staining (n = 6) or immunohistochemical staining for DNAJB9 (n = 1). Among 29

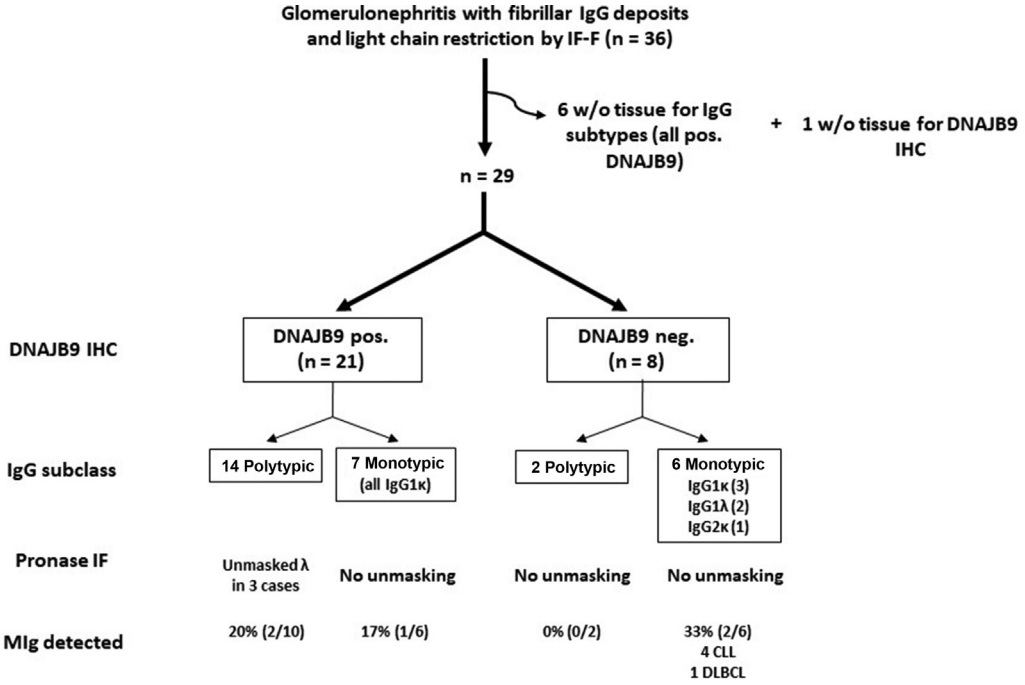

Figure 1. Case inclusion criteria and diagnostic algorithm. DNAJB9 immunostain, pronase immunofluorescence (IF-P), and IgG subtype staining by IF were performed on 29 biopsy samples from 28 patients showing glomerulonephritis with fibrillar IgG deposits and light chain restriction by frozen immunofluorescence (IF-F). CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; IF, immunofluorescence; IHC, immunohistochemistry; MIg, monoclonal immunoglobulin; neg, negative; pos, positive; w/o, without.

remaining biopsy samples from 28 patients, 93% (27 of 29) showed κ-restriction and 7% (2 of 29) showed λ-restriction by IF-F. These biopsy samples were classified (Figure 1) on the basis of immunohistochemical staining for DNAJB9 (a marker of fibrillary glomerulonephritis [FGN]), pronase immunofluorescence (IF-P) staining for κ and λ, and IgG subtype staining into 4 distinct categories: (i) DNAJB9-positive with polytypic IgG deposits (i.e., polytypic FGN, pFGN); (ii) DNAJB9-positive with monotypic IgG deposits (i.e., monotypic FGN, mFGN); (iii) DNAJB9-negative with polytypic IgG deposits; and (iv) DNAJB9-negative with monotypic IgG deposits. Monotypic deposits were defined as staining for a single IgG subtype, light chain restricted by IF-F and failure to unmask the reciprocal light chain by IF-P.

DNAJB9-Positive With Polytypic IgG Deposits (i.e., pFGN)

A total of 14 biopsy samples (48%) were classified as having DNAJB9-positive polytypic IgG deposits (pFGN) (Table 1). By IF-F, 13 were κ-restricted and 1 was λ–restricted. The IF-P unmasked staining for λ light chain in 25% (3 of 12). Polytypia was demonstrated by staining for multiple IgG subtypes in all 14 biopsy samples, 13 (93%) of which stained for IgG1 and IgG4. Extraglomerular deposits, when present (n = 6), showed the same pattern of IgG subtype staining as glomeruli. Congo red stain, performed in 13 cases, was uniformly negative.

A total of 14 patients with pFGN had a median age of 64.5 years, and included 5 male and 9 female individuals. Among 10 patients with reported self-identified race, 8 (80%) were White, 1 was Black, and 1 was of Hispanic descent. In all, 12 (86%) had hypertension and 6 (43%) had diabetes. One patient had a history of MGUS (with serum IgGκ and IgGλ M-spikes); none had overt plasma cell neoplasia, B-cell lymphoma, or autoimmune disease.

Patients with pFGN presented with a median serum creatinine of 2.4 mg/dl (range, 1.1–4.2 mg/dl), urine protein-to-creatinine ratio (or 24-hour urine protein) 3.9 g/g or g/day (range, 0.84–10 g/g), and a median serum albumin 3.4 g/dl (range, 2.7–4.4 g/dl). Ten (71%) had hematuria and 5 (36%) had edema. Serologic evaluation was significant for 2 with positive antinuclear antibody and 2 with hepatitis C virus infection. None had positive serologies for hepatitis B. With the exception of the single patient with known MGUS, 10 patients had serum protein electrophoresis performed, including 9 with urine protein electrophoresis, and all were negative for M-protein at the time of presentation.

Light microscopy showed glomerulonephritis with mesangial proliferative (n = 11), membranoproliferative (n = 1), diffuse proliferative (n = 1), and diffuse sclerosing (n = 1) patterns. The median percentage of global glomerulosclerosis was 8% (range, 3%–41%),

Table 1. Results of DNAJB9 IHC, IF-F, IgG subtype staining, IF-P, and Congo red

	IF-F[a]						IF-P[a]				
Pt	IgG	C3	C1	κ	Λ	IgG subtype (intensity)	Unmasked reciprocal light chain?	Pronase IgG	Pronase κ	Pronase λ	Congo red positive?
DNAJB9-positive polytypic (FGN)											
1	3	2	1	3	+/–	IgG1 (2), IgG4 (2)	NA		NA		N
2	3	3	1	0	2	IgG1 (3), IgG2 (+/–)	N	2	0	2	N
3	3	+/–	0	3	+/–	IgG1 (+/–), IgG4 (3)	N	2	2	0	N
4	3	1	2	3	0	IgG1 (2), IgG4 (3)	N	3	3	0	N
5	3	1	0	3	+/–	IgG1 (2), IgG4 (2)	N	1	0	1	N
6	1	0	0	1	0	IgG1 (+/–), IgG4 (+/–)	N	1	1	0	N
7	3	2	0	2	0	IgG1 (2), IgG2 (+/–), IgG3 (+/–), IgG4 (3)	Y	1	1	1	N
8	2	2	1	2	+/–	IgG1 (1), IgG4 (2)	NA		NA		N
9	3	2	+/–	3	+/–	IgG1 (3), IgG4 (3)	N	2	2	1	N
10	3	2	0	3	0	IgG1 (3), IgG2 (+/–), IgG4 (3)	Y	3	3	1	N
11	3	3	1	3	0	IgG1 (3), IgG4 (2)	N	3	3	0	N
12	2	2	0	2	0	IgG1 (3), IgG2 (2), IgG4 (2)	N	3	2	0	N
13	3	2	+/–	3	0	IgG1 (2), IgG4 (2)	N	3	3	0	N
14	3	3	0	2	0	IgG1 (3), IgG4 (2)	Y	3	3	3	NA
DNAJB9-positive monotypic (mFGN)											
1a[b]	3	2	1	2	0	IgG1 (3)	N	3	3	0	N
1b[b]	3	3	1	3	0	IgG1 (2)	N	+/–	+/–	0	N
2	2	2	0	1	0	IgG1 (2)	N	+/–	+/–	0	N
3	2	2	0	2	0	IgG1 (2)	NA		NA		N
4	3	2	0	3	0	IgG1 (3)	N	2	1	0	N
5	2	+/–	0	3	0	IgG1 (3)	N	3	3	0	N
6	2	2	+/–	2	0	IgG1 (2)	N	NA	2	0	N
DNAJB9-negative polytypic											
1	1	0	0	1	0	IgG1 (2), IgG4 (1)	N	0	0	0	N
2	1	2	0	0	1	IgG1 (2), IgG3 (1)	N	0	0	0	N
DNAJB9-negative monotypic											
1	2	2	+/–	2	0	IgG2 (3)	N	2	1	0	N
2	3	2	2	0	3	IgG1 (3)	N	3	0	3	N
3	3	2	3	3	0	IgG1 (3)	NA		NA		NA
4	3	3	0	3	0	IgG1 (2)	NA		NA		N
5	3	2	+/–	2	0	IgG1 (3)	N	2	2	0	N
6	3	2	0	0	3	IgG1 (3)	N	3	0	3	N

FGN, fibrillary glomerulonephritis; IF, immunofluorescence; IF-F, frozen immunofluorescence; IF-P, pronase immunofluorescence; mFGN, monoclonal fibrillary glomerulonephritis; N, no; NA, not available; Y, yes.
[a]Immunofluorescence intensity was graded on a scale of 0, +/–, 1–3.
[b]These cases represent 2 biopsy samples from the same patient.

median tubular atrophy and interstitial fibrosis was 50% (range, 0%–75%), and moderate-to-severe vascular sclerosis was present in 10 patients (71%). None had involvement of the kidney by lymphoma or myeloma. Ultrastructural examination revealed randomly oriented, nonbranching fibrillar deposits embedded in the matrix of the mesangium and glomerular basement membranes. The fibrils had mean diameter of 17 to 22 nm and lacked hollow cores. Podocytes exhibited 40% to 100% foot process effacement.

Among 9 patients (64%) with available follow-up (median, 1.5 years; range, 1–7 years), 1 with MGUS on presentation continued to have detectable monoclonal serum IgGκ and IgGλ M-spikes and monoclonal urine IgGκ with free λ light chain. One patient developed an IgGκ and a free λ band on SIFE and UIFE. No patients developed overt hematolymphoid malignancy.

DNAJB9-Positive With Monotypic IgG Deposits (i.e., mFGN)

Seven biopsy samples (24%, 7 of 29) were classified as having DNAJB9-positive monotypic deposits (mFGN), including a sample from 1 patient with a repeat biopsy (Table 1). All were κ restricted by IF-F. Use of IF-P failed to unmask λ light chain in 6 biopsy samples tested (Figure 2). All showed glomerular IgG1 restriction by IgG subtype staining (Figure 2). Extraglomerular deposits, present in 2 biopsy samples, also showed IgG1 subtype restriction. Congo red stain was negative in all 7 cases.

Clinical features are described in Table 2. The median age was 70.5 years (range, 50–79 years). All patients self-identified as White and 4 were female. Five (83%) had hypertension and 4 (67%) had diabetes mellitus. One patient had a history of IgGκ MGUS; none had overt plasma cell neoplasia, B-cell lymphoma, or autoimmune disease.

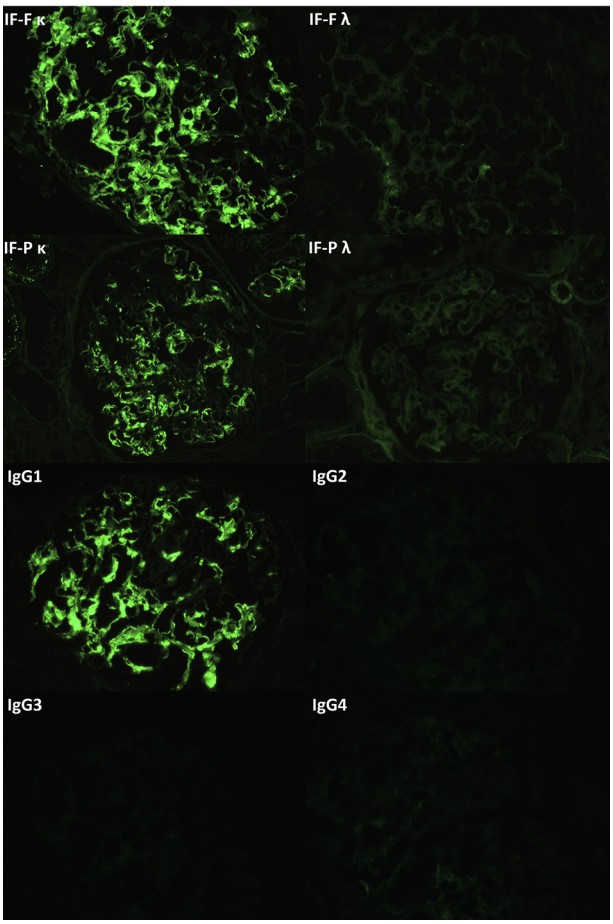

Figure 2. Representative immunofluorescence findings from a patient with DNAJB9-positive monotypic fibrillary glomerulonephritis. Frozen immunofluorescence (IF-F) revealed 3+ smudgy staining for κ without significant staining for λ. Pronase immunofluorescence (IF-P) showed strong smudgy staining for κ and failed to unmask staining for λ. The deposits showed strong staining for IgG1, but not IgG2, IgG3, or IgG4. Taken together, the IF findings supported a diagnosis of fibrillary glomerulonephritis with monotypic IgG1-κ deposits (immunofluorescence microscopy, original magnification ×200).

On initial presentation, the median serum creatinine was 2.9 mg/dl (range, 1.2–7.5 mg/dl), urine protein-to-creatinine ratio (or 24-hour urine protein) was 1.7 g/g or g/d (range, 1.1–6.9 g/g), and median serum albumin was 3.5 g/dl (range 2.5-4.2 g/dl). Four patients (67%) had hematuria and 2 (33%) had edema. Although no patient had full nephrotic syndrome at initial presentation, 1 patient later developed full nephrotic syndrome. Serologic evaluation revealed 3 patients with low-titer antinuclear antibody and 2 with positive p-antineutrophil cytoplasmic antibody (ANCA) or myeloperoxidase antibody. None had positive serologies for hepatitis B or C. One patient (patient 3) with a history of MGUS had an IgGκ paraprotein identified by SIFE. One patient (patient 5) had an elevated serum free light chain (FLC) ratio in the setting of renal failure.

Pathologic features are described in Table 3. Light microscopy showed glomerulonephritis with mesangial proliferative (n = 2), membranoproliferative (n = 2), and diffuse sclerosing (n = 1) patterns. Two cases showed necrotizing and crescentic glomerulonephritis with mild mesangial proliferative features, both of which were associated with ANCA seropositivity. The median percentage of global glomerulosclerosis was 25% (range, 4%–58%), median tubular atrophy and interstitial fibrosis was 40% (range, 0–65%), and moderate-to-severe vascular sclerosis was present in all patients. Three patients had coexisting diabetic nephropathy, and none had involvement of the kidney by lymphoma or myeloma. Ultrastructural examination most commonly revealed randomly oriented, nonbranching fibrillar deposits embedded in the matrix of the mesangium and glomerular basement membranes with mean diameter of 18 to 35 nm. The fibrils lacked hollow cores and focally coexisted with amorphous electron-dense deposits in 6 of 7 biopsy samples (86%). Podocytes exhibited 30% to 90% foot process effacement.

The median follow-up (Table 2) was 6 months (range, 2–36 months). No patient was found to have plasma cell or B-cell neoplasia. Two patients were treated with rituximab (including 1 combined with cyclophosphamide and 1 combined with corticosteroids), 1 with corticosteroids only, and 1 with adrenocorticotropic hormone only. One patient did not receive any treatment. Two patients developed end-stage renal disease and died. Of the remaining 4 patients, 3 had persistent renal insufficiency (i.e., serum creatinine >1.3 mg/dl), and 2 had increase in proteinuria.

DNAJB9-Negative With Polytypic IgG Deposits

Two biopsy samples (7%, 2 of 29) were classified as having DNAJB9-negative polytypic IgG deposits (Table 1). By IF-F, 1 was κ restricted and 1 was λ restricted. By IgG subtype staining, 1 showed IgG1 and IgG4 deposits and 1 showed IgG1 and IgG3 deposits. Use of IF-P failed to unmask the reciprocal light chain in either biopsy sample. Congo red staining was negative. Neither patient had evidence of monoclonal gammopathy or hematolymphoid malignancy.

DNAJB9-Negative With Monotypic IgG Deposits

Six biopsy samples (21%, 6 of 29) were classified as having DNAJB9-negative monotypic deposits (Table 1). By IF-F, 4 were κ restricted and 1 was λ restricted. Use of IF-P failed to unmask the reciprocal light chain in 4 biopsy samples tested. According to IgG subtype staining, the 6 monotypic biopsy samples included 3

Table 2. Clinical features of patients with monotypic fibrillary glomerulonephritis

				Renal parameter						Past medical history				
Pt	Age (yr)	Sex	Race	SCr (mg/dl)	UPCR (g/g)	Albumin (g/dl)	Edema	NS	Hematuria	HTN	DM	Autoimmmune disease	MGUS or myeloma	Lymphoma
1[a]	50	F	W	2.2	1.4	3.7	Y	N		Y	Y	N	N	N
2	79	M	W	2.3	6.9	3	N	N	N	Y	Y	N	N	N
3	67	F	W	7.5	1.4		Y		Y	Y	Y	N	MGUS	N
4	74	F	W	3.4	5	2.5	N	N	Y	Y	Y	N	N	N
5	78	F	W	4.4	1.9	3.5	N	N	Y	Y	N	N	N	N
6	56	M	W	1.2	1.1	4.2	N	N	Y	N	N	N	N	N

	Laboratory values								Follow-up						
	ANA	ANCA	Low C3/ C4	+ HBsAg	+ HCV Ab	SPEP	UPEP	Serum FLC ratio	Duration (mo)	Treatment	Deceased	ESRD	SCr (mg/dl)	UPCR	Albumin (g/dl)
1[a]	Y (1:80)	N	N	N	N	N		Normal	36	RTX and CYC	N	N	2.7	7.4	3.4
2	Y (1:40)		N						6	None	N	N	2.6	8.2	
3	N	Y (p-ANCA)	N	N	N	IgGκ			32	CS	Y	Y	3.4	0.1	1.5
4	N	Y (MPO)	N	N	N	N	N		5	RTX + CS	N	N	1.9	5	2.6
5	N	N	N	N	N	N	N	Elevated	2		Y	Y	5.7		3.2
6	N	N	N	N	N	N	N	Normal	6	ACTH	N	N	0.6	1.0	4.5

ACTH, adrenocorticotropic hormone; ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibody; CS, corticosteroids; CYC, cyclophosphamide; DM, diabetes mellitus; ESRD, end-stage renal disease; FLC, free light chain; HBsAg, hepatitis B surface antigen; HCV Ab, hepatitis C antibody; HTN, hypertension; MGUS, monoclonal gammopathy of unknown significance; MPO, myeloperoxidase; N, no/negative; NS, nephrotic syndrome; Pt, patient; UPCR, urine protein-to-creatinine ratio (or 24-hr urine protein); UPEP, urine protein electrophoresis; SCr, serum creatinine; SPEP, serum protein electrophoresis; RTX, rituximab; W, White; Y, yes.
[a]Patient 1 had 2 biopsy samples.

IgG1κ, 2 IgG1λ, and 1 IgG2κ. Congo red staining was negative.

Clinical features are presented in Table 4. The median age was 70 years (range, 54–82 years). Four patients were female, and the cohort included 4 self-identified White patients and 1 each of self-identified Asian and Hispanic descent. Four (67%) had hypertension and 1 (17%) had diabetes mellitus. Four (67%) had chronic lymphocytic leukemia (CLL), 3 of which cases were diagnosed prior to kidney biopsy. Two had a history of autoimmune disease, including 1 with hypothyroidism and 1 with Sjögren's syndrome. None had plasma cell neoplasia.

Table 3. Pathologic features of patients with monotypic fibrillary glomerulonephritis

	Light microscopy						Immunofluorescence microscopy		
Pt	Predominant LM pattern	% Globally sclerotic glomeruli	% IFTA	Vascular sclerosis[a]	Congo red positive	Coexisting diagnosis	Positive immunoreactant (intensity)	Positive IgG subclass (intensity)	Unmasking by pronase IF
1a[b]	MPGN	44	65	Moderate	N		IgG (3), C3 (2), C1 (1), κ (2)	IgG1 (3)	N
1b[b]	MPGN	20	60	Moderate	N		IgG (3), C3 (3), C1 (1), κ (3)	IgG1 (2)	N
2	MesGN	10	40	Severe	N	NDGS	IgG (2), C3 (2), κ (1)	IgG1 (2)	N
3	NCGN	29	40	Severe	N	DDGS	IgG (2), C3 (2), κ (2)	IgG1 (2)	
4	NCGN	20	10	Moderate	N	DDGS	IgG (3), C3 (2), κ (3)	IgG1 (3)	N
5	DSGN	58	60	Severe	N		IgG (2), C3 (+/–), κ (3)	IgG1 (3)	N
6	MesGN	7	0	Moderate	N		IgG (2), C3 (2), C1 (+/–), κ (2)	IgG1 (2)	N

	Electron microscopy								
	Mesangial deposits	Subendothelial deposits	Subepithelial deposits	Intramembranous deposits	Mean diameter of the fibrils (nm)	Range of diameters (nm)	Hollow core	Amorphous deposits	FPE
1a[b]	Y	N	N	Y	25	18 to 38	N	Y	80
1b[b]	Y	N	N	Y	25	18 to 38	N	Y	90
2	Y	N	N	Y	18	15 to 24	N	N	90
3	Y	N	N	N	25	18 to 30	N	Y	30
4	Y	N	N	Y	35	30 to 42	N	Y	80
5	Y	N	N	Y	24	14 to 30	N	Y	60
6	Y	N	Y	Y	24	16 to 30	N	Y	60

DDGS, diffuse diabetic glomerulosclerosis; DSGN, diffuse sclerosing; FPE, foot process effacement; GN, glomerulonephritis; IF, immunofluorescence; IFTA, interstitial fibrosis and tubular atrophy; LM, light microscopic; MesGN, mesangial proliferative GN; MPGN, membranoproliferative GN; N, no; NCGN, necrotizing and crescentic GN; NDGS, nodular diabetic glomerulosclerosis; Pt, patient; Y, yes.
[a]These cases represent 2 biopsy samples from the same patient.
[b]Defined as the maximum of arteriosclerosis or arteriolosclerosis.

Table 4. Clinical features of patients with glomerulonephritis with DNAJB9-negative monotypic IgG deposits

				Renal parameter						Past medical history				
Pt	Age (yr)	Sex	Race	SCr (mg/dl)	UPCR (g/g)	Albumin (g/dl)	Edema	NS	Hematuria	HTN	DM	Autoimmmune disease	MGUS or myeloma	Lymphoma
1	82	F	W	1.8	8	3.1	Y	Y	N	Y	Y	N	N	CLL × 37 yr
2	70	M	W	2.5	3.2				N	N	N	N	N	CLL × 10 yr
3	64	M	A	2.1	12	3	Y	Y	N	Y	N	N	N	CLL
4	70	F	W	1.5	6	3	Y	Y	Y	Y	N	N	N	CLL × 8 yr
5	54	F	H	0.8	1		N	N	Y	N	N	Sjögren	N	HIV-associated DLBCL
6	78	F	W	0.7	4	3	Y	Y	N	Y	N	Hypothyroidism	N	N

	Laboratory values								Follow-up						
	ANA	ANCA	Low C3/ C4	+ HBsAg	+ HCV Ab	SPEP	UPEP	Serum FLC ratio	Duration (mo)	Treatment	Deceased	ESRD	SCr (mg/dl)	UPCR	Albumin (g/dl)
1		N	N	N	N	Small spike		Elevated	28	None	Y	Y			
2															
3	N	N	C3	N	N	N	N	Elevated	52	RTX	N	N	1.4	0.4	4.2
4	N	N	N	N	N	IgGκ	N		<1mo		N	N	1.1	6	3
5	Y (1:320)	N	C4	N	N				38	ART and R-CHOP	N	N	0.7	0.1	Normal
6	N	N	N	N	N	N	N	Normal	11	CYC, RTX, CS	N	N	0.9	6	3.3

A, Asian; ANA, antinuclear antibody; ANCA, antineutrophil cytoplasmic antibody; ART, antiretroviral therapy; W, White; CLL, chronic lymphocytic leukemia/lymphoma; CS, corticosteroids; CYC, cyclophosphamide; DM, diabetes mellitus; DLBCL, diffuse large B-cell lymphoma; ESRD, end-stage renal disease; FLC, free light chain; H, Hispanic; HAART, highly active antiretroviral therapy; HBsAg, hepatitis B surface antigen; HCV Ab, hepatitis C antibody; HTN, hypertension; MGUS, monoclonal gammopathy of unknown significance; N, no; NS, nephrotic syndrome; Pt, patient; R-CHOP, rituximab, cyclophosphamide, doxorubicine, vincristine, prednisone, RTX, rituximab; SCr, serum creatinine; SPEP, serum protein electrophoresis; UPCR, urine protein-to-creatinine ratio (or 24-hr urine protein); UPEP, urine protein electrophoresis; W, White; Y, yes.

The median serum creatinine at presentation was 1.7 mg/dl (range, 0.7–2.5 mg/dl), median urine protein-to-creatinine ratio (or 24-hour urine protein) was 5 g/g or g/d (range, 1–12 g/g), and median serum albumin was 3 g/dl (range, 3–3.1 g/dl). Four patients had edema and full nephrotic syndrome. Serologic evaluation revealed 1 patient with positive antinuclear antibody and 2 with low serum complements. None had positive ANCA or hepatitis B or C serologies. Three had a high serum FLC ratio and 2 had serum protein electrophoresis with detectable M-protein, 1 of which was IgGκ (in a patient with glomerular immunofluorescence staining for IgG1κ).

Pathologic features are presented in Table 5. Light microscopy demonstrated membranous (n = 4), focal endocapillary proliferative (n = 1), and membranoproliferative (n = 1) patterns. The median percentage of global glomerulosclerosis was 15% (range, 0% to 36%), median tubular atrophy and interstitial fibrosis was 28% (range, 0%–50%), and mild vascular sclerosis was present in all but 1 case. Two patients showed infiltration of the kidney parenchyma by CLL. Ultrastructural examination uniformly demonstrated nonbranching, randomly oriented fibrils with a mean diameter ranging from 16 to 28 nm embedded in the matrix of the mesangium (Figure 3). In cases with a membranous pattern, the subepithelial fibrillar deposits displayed limited permeation of the outer aspect of the glomerular basement membrane matrix (Figure 3). The deposits lacked hollow cores at ×50,000, and higher magnification (up to ×100,000) also failed to reveal hollow cores (Figures 3 and 4). The fibrils at least focally coexisted with amorphous electron dense deposits in 6 of 7 cases (86%). In some foci,

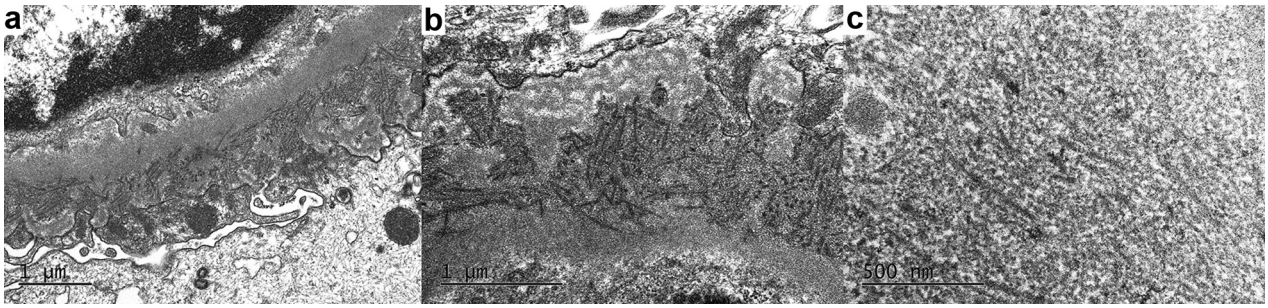

Figure 3. Representative electron microscopy findings from a patient (patient 6) with "glomerulonephritis with monotypic DNAJB9-negative fibrillar IgG deposits." (a) Electron microscopy shows randomly oriented, nonbranching deposits with fibrillary substructure in mesangial distribution (not shown) and subepithelial distribution (electron microscopy, original magnification ×30,000). (b) The fibrils had a mean diameter of 28 nm and showed limited permeation of the outer portion of the glomerular basement membrane matrix (electron microscopy, original magnification ×50,000). (c) High-power examination failed to reveal hollow cores typical of immunotactoid glomerulonephritis (electron microscopy, original magnification ×100,000).

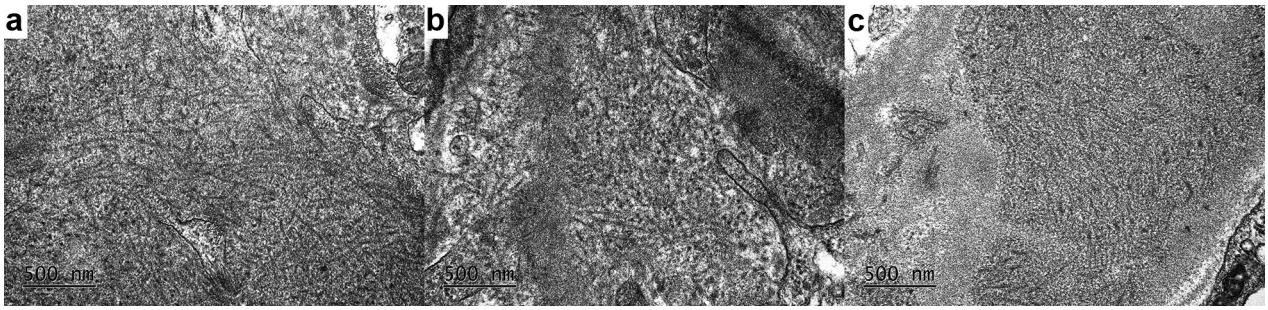

Figure 4. Representative electron microscopy images from patients with (a) polytypic fibrillary glomerulonephritis, (b) monotypic fibrillary glomerulonephritis, and (c) "glomerulonephritis with monotypic DNAJB9-negative fibrillar deposits" shown at the same magnification. Ultrastructural examination revealed nonbranching, largely randomly oriented fibrils embedded within the mesangial matrix, with a mean fibril diameter of 22, 24, and 28 nm, respectively. Note the absence of hollow cores in image c (electron microscopy, original magnification ×50,000).

fibrils exhibited vaguely parallel arrangement (Figure 5).

Follow-up (Table 4) was available for 5 patients (83%), with a median follow-up duration of 28 months (range <1 month to 52 months). In addition to 4 patients with CLL, 1 patient was found to have HIV infection and diffuse large B-cell lymphoma following the biopsy. One had no known history of hematolymphoid neoplasm. Three (50%) were treated with rituximab, including 1 with R-CHOP (rituximab, cyclophosphamide, doxorubicine, vincristine, prednisone) and antiretroviral therapy, and 1 with steroids and cyclophosphamide. Two of the treated patients achieved remission of proteinuria to <1 g/g or g/d. One received no treatment, progressed to end-stage renal disease, and died.

DISCUSSION

We performed a systematic workup of 29 biopsy samples showing glomerulonephritis with fibrillar IgG deposits and light chain restriction by IF-F. We found DNAJB9 immunostain and IgG subtype staining to be most useful in classifying these entities into 4 diagnostically distinct diagnostic categories (Figure 1).

In all, 72% of biopsy samples in our cohort demonstrated positive staining for DNAJB9, a marker of FGN.[5,13] Most FGN exhibits polytypic IgG staining by IF-F. Over the time period of this study, 12% (21 of 172) of biopsy samples diagnosed as FGN in our practice exhibited light chain–restricted staining by IF-F (20 κ and 1 λ), in line with recent reports.[7,9] After IF-P and IgG subtype staining, only 4% (7 of 172) of biopsies were classified as mFGN. Given the potential therapeutic implications of the current expert recommendations to include mFGN in the spectrum of MGRS,[8] the inclusion of IF-P and IgG subtype staining to define truly "monotypic" deposits is of paramount importance.

In our cohort, IF-P failed to unmask staining for the reciprocal light chain in 83% of FGN with light chain restriction by IF-F. As such, IF-P was less reliable in excluding mFGN than in a recent series reported by Said *et al.*,[9] in which IF-P showed staining for both light chains in 43% (15 of 35). It is noteworthy that our cohort was enriched for κ-restricted cases. Said *et al.* were able to unmask staining for λ light chain by IF-P in only 7% of cases that exhibited κ restriction by IF-F, in line with our experience.[9] Taken together, these findings suggest that IF-P may be insufficient to exclude polytypic deposits in cases of FGN with κ-restricted staining by IF-F. In our experience, IgG subtype staining was more useful to exclude monotypic deposits, revealing staining for ≥2 IgG subtypes (usually IgG1 and IgG4) in 67% of biopsy samples, including 9 that would have been misclassified as monotypic by IF-F and IF-P alone.

After IF-F, IF-P, and IgG subtype staining, 7 biopsy samples from 6 patients met criteria for mFGN. One patient had a history of IgGκ MGUS. This patient's biopsy showed predominantly features of ANCA-

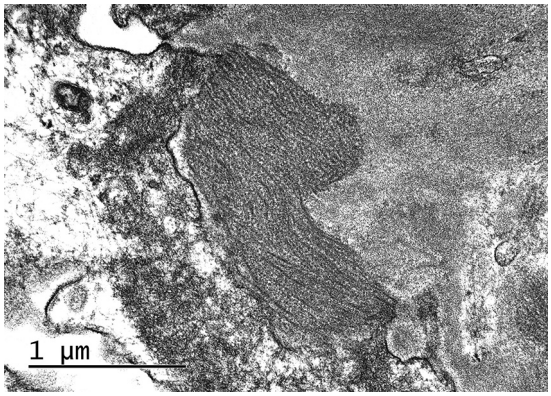

Figure 5. An example of focal parallel alignment of fibrils in a patient with "glomerulonephritis with monotypic DNAJB9-negative fibrillar IgG deposits." Note the absence of hollow cores (electron microscopy, original magnification ×50,000).

Table 5. Pathologic features of patients with glomerulonephritis with DNAJB9-negative monotypic IgG deposits

	Light microscopy						Immunofluorescence microscopy		
Pt	Predominant LM Pattern	% Globally sclerotic glomeruli	% IFTA	Vascular sclerosis[a]	Congo red positive	Coexisting diagnosis	Positive immunoreactant (intensity)	Positive IgG subclass (intensity)	Unmasking by pronase IF
1	MGN (PLA2R NOS)	10	50	Mild	N	N	IgG (2), C3 (2), C1 (+/–), κ (2)	IgG2 (3)	N
2	FPGN	36	40	Moderate	N	CLL involvement	IgG (3), C3 (2), C1 (2), λ (3)	IgG1 (3)	N
3	MPGN	21	25	Mild		CLL involvement	IgG (3), C3 (3), C1 (3), κ (3)	IgG1 (3)	
4	MGN (PLA2R-neg)	10	20	Mild	N	N	IgG (2), C3 (2), C1 (+/–), κ (2)	IgG1 (3)	N
5	MGN (PLA2R-neg)	0	30	Mild	N	N	IgG (3), C3 (3), κ (3)	IgG1 (2)	
6	MGN (PLA2R-neg)	20	0	Mild	N	N	IgG (3), C3 (2), λ (3)	IgG1 (3)	N

	Electron microscopy								
	Mesangial deposits	Subendothelial deposits	Subepithelial deposits	Intramembranous deposits	Mean diameter of the fibrils	Range of diameters	Hollow core	Amorphous deposits	FPE
1	Y	N	Y	Y	22	16 to 30	N	Y	90
2	Y	N	Y	N	16	14 to 21	N	Y	50
3	Y	Y	Y	N	25	20 to 38	N	Y	80
4	Y	N	Y	Y	26	18 to 32	N	Y	100
5	Y	N	Y	N	24	19 to 30	N	Y	90
6	Y	N	Y	Y	28	22 to 35	N	N	90

DDGS, diffuse diabetic glomerulosclerosis; FPGN, focal endocapillary proliferative GN; FPE, foot process effacement; GN, glomerulonephritis; IF, immunofluorescence; IFTA, interstitial fibrosis and tubular atrophy; LM, light microscopic; MesGN, mesangial proliferative GN; MN, membranous GN; MPGN, membranoproliferative GN; N, no/negative; NDGS, nodular diabetic glomerulosclerosis; NCGN, necrotizing and crescentic GN; Pt, patient; Y, yes.
[a]Defined as the maximum of arteriosclerosis or arteriolosclerosis.

mediated necrotizing and crescentic GN, with coincidental mesangial proliferative IgG1κ mFGN. One patient had an elevated sFLC ratio in the setting of acute kidney injury, but no detectable monoclonal protein in the serum or urine. The remaining 4 patients had no detectable monoclonal protein. None had evidence of overt hematolymphoid malignancy. The incidence of dysproteinemia did not vary significantly between pFGN and mFGN (20% and 17%, respectively). These findings, together with those recently reported by Said *et al.*,[9] bring into question the inclusion of mFGN within the spectrum of MGRS.

Six cases (21%) were characterized as glomerulonephritis with DNAJB9-negative monotypic IgG deposits. These cases had several clinical and pathologic features in common with immunotactoid glomerulonephritis, namely, frequent association with CLL, frequent membranous pattern on light microscopy, frequent IgG1 subtype restriction, focal parallel alignment of fibrils, and limited permeation of the glomerular basement membrane matrix.[2,4,11,14] The absence of detectable hollow cores is likely due to the small size of the fibrils (overlapping with the range of FGN). We believe that these cases most likely fall within the spectrum of atypical immunotactoid glomerulonephritis. Similar cases likely have been variably classified in the literature.[2–4,11] Organization of monoclonal immunoglobulins depends on multiple factors, including physicochemical properties related to the specific amino acid sequence of the variable regions, glycosylation patterns, concentration, purity, and tissue microenvironment.[15,16] The presence of monotypic deposits with organized substructure but lacking DNAJB9 staining, whether labeled as fibrils or microtubules, should be taken as a feature that correlates with a higher likelihood of underlying hematolymphoid neoplasm, particularly CLL.

There are several limitations to this study. Because of the retrospective and descriptive nature of these studies, clinical evaluation, diagnosis, and treatment were not standardized. A minority of the patients were lost to follow-up. Archived tissue was not available for ancillary studies in several cases. Finally, we could not perform liquid chromatography–mass spectrometry to further analyze the glomerular deposits.

In conclusion, we present a systematic diagnostic approach to glomerulonephritis with fibrillar IgG deposits and apparent light chain restriction by IF-F. Ancillary studies including DNAJB9 immunostain, IF-P, and IgG-subtype staining allowed more precise subclassification in all cases. Most cases that would have been classified as monotypic FGN by IF-F alone were reclassified as polytypic FGN, largely on the basis of IgG subtype staining. Monotypic FGN was not associated with dysproteinemia in most patients, and was not associated with overt hematolymphoid neoplasm in any patient, casting doubt on its inclusion in the spectrum of MGRS-related lesions. In contrast, DNAJB9-negative

glomerulonephritis with monotypic fibrillar IgG deposits by all modalities exhibited many features reminiscent of ITG, including focally parallel fibril alignment and a strong association with CLL, but lacked definitive hollow cores (i.e., *bona fide* microtubules). Our findings reveal a more complex spectrum of nonamyloid fibrillar deposition disorders than previously recognized.

DISCLOSURE

All the authors declared no competing interests.

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