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CLINICAL RESEARCH

DNAJB9 Is a Specific Immunohistochemical Marker for Fibrillary Glomerulonephritis

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Introduction: Fibrillary glomerulonephritis (FGN) is a rare disease with unknown pathogenesis and a poor prognosis. Until now, the diagnosis of this disease has required demonstration of glomerular deposition of randomly oriented fibrils by electron microscopy that are Congo red negative and stain with antisera to Igs. We recently discovered a novel proteomic tissue biomarker for FGN, namely, DNAJB9.

Methods: In this work, we developed DNAJB9 immunohistochemistry and tested its sensitivity and specificity for the diagnosis of FGN. This testing was performed on renal biopsy samples from patients with FGN (n = 84), amyloidosis (n = 21), a wide variety of non-FGN glomerular diseases (n = 98), and healthy subjects (n = 11). We also performed immunoelectron microscopy to determine whether DNAJB9 is localized to FGN fibrils.

Results: Strong, homogeneous, smudgy DNAJB9 staining of glomerular deposits was seen in all but 2 cases of FGN. The 2 cases that did not stain for DNAJB9 were unique, as they had glomerular staining for IgG only (without κ or λ) on immunofluorescence. DNAJB9 staining was not observed in cases of amyloidosis, in healthy subjects, or in non-FGN glomerular diseases (with the exception of very focal staining in 1 case of smoking-related glomerulopathy), indicating 98% sensitivity and > 99% specificity. Immunoelectron microscopy showed localization of DNAJB9 to FGN fibrils but not to amyloid fibrils or immunotactoid glomerulopathy microtubules.

Conclusion: DNAJB9 immunohistochemistry is sensitive and specific for FGN. Incorporation of this novel immunohistochemical biomarker into clinical practice will now allow more rapid and accurate diagnosis of this disease.

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KEYWORDS: biomarker; DNAJB9; fibrillary glomerulonephritis; immunoelectron microscopy; immunohistochemistry; kidney biopsy

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Fibrillary glomerulonephritis (FGN) is a rare disease that was first described in the literature by Rosenmann and Eliakim in 1977[1] and was later recognized as a distinct glomerular disease by Duffy *et al*. in 1983.[2] Current diagnostic criteria for FGN require the demonstration of haphazardly arranged, straight fibrils measuring 10 to 30 nm in thickness in the mesangium and/or along the glomerular basement membranes by electron microscopy (EM).[3–6] On immunofluorescence (IF), in most cases, the deposits stain for IgG, both κ and λ light chains, and C3. The majority of cases show IgG4 subtype restriction. On light microscopy (LM), most cases exhibit mesangial expansion/hypercellularity, with or without duplication of the glomerular basement membranes. In the vast majority of cases of FGN, these deposits are Congo red negative, which is important in distinguishing FGN from renal amyloidosis.

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There are several limitations of the current means of diagnosing FGN. First, no single feature on LM, IF, or EM is pathognomonic of this disease. The LM finding of mesangial expansion and hypercellularity can be lacking in the early stage of disease and can also be seen in several other glomerular diseases such as IgA nephropathy, immunotactoid glomerulopathy, diabetic glomerulosclerosis, fibronectin glomerulopathy, and collagenofibrotic glomerulopathy. The IF findings can overlap with other forms of immune-mediated glomerulonephritis (such as lupus nephritis and membranous glomerulonephritis), with immunotactoid glomerulopathy, and with renal amyloidoses (including heavy and light chain amyloidosis, heavy chain amyloidosis, and AA amyloidosis with entrapped Igs). On EM, FGN can be difficult to distinguish from other glomerular diseases that exhibit fibrils or small microtubules, including amyloidosis, immunotactoid glomerulopathy associated with chronic lymphocytic leukemia, diabetic fibrillosis, and fibronectin glomerulopathy. Second, kidney biopsy samples may be limited, without glomeruli available for IF or EM. In these situations, a pathologic diagnosis of FGN is not possible without repeat biopsy. Third, because EM is labor intensive and expensive, it is not routinely performed in many pathology laboratories, particularly in the developing countries, and hence FGN is likely an underdiagnosed disease.

Through the use of laser microdissection–assisted liquid chromatography–tandem mass spectrometry (LMD/MS-MS), we recently discovered a novel proteomic biomarker for FGN: DnaJ homolog subfamily B member 9 (DNAJB9), a member of the molecular chaperone gene family.[7] In this work, we developed DNAJB9 immunohistochemistry (IHC) and tested its sensitivity and specificity for the diagnosis of FGN in a large cohort of patients. We also performed immunoelectron microscopy (immuno-EM) to determine whether DNAJB9 is localized to FGN fibrils.

MATERIALS AND METHODS

Study Patients

All FGN cases included in this study fulfilled the following previously established diagnostic criteria[5]: glomerular deposition of fibrils that were (i) randomly oriented, (ii) lacked hollow centers at magnification of <30,000; (iii) were Congo-red negative; and (iv) stained with antisera to Igs by IF. Table 1 shows the salient clinical characteristics at diagnosis and pathologic findings of the 84 Mayo Clinic FGN cases. The pathologic diagnoses of non-FGN glomerular diseases (NFGNGDs) and amyloidosis were made using current standard pathologic diagnostic criteria. Three of the FGN cases were included in our previously published clinicopathologic series on FGN.[5]

Renal Biopsy Sample Evaluation

Standard processing of renal biopsy samples included LM, IF, and transmission EM. For LM, all renal biopsy samples were stained with hematoxylin and eosin, periodic acid–Schiff, Masson's trichrome, and Jones methenamine silver. All cases of FGN and amyloidosis were stained with Congo red. For IF, 4-μm cryostat sections were stained with polyclonal fluorescein isothiocyanate–conjugated antibodies to IgG, IgM, IgA, C3, C1q, κ, and λ. In cases that lacked glomeruli in the frozen tissue, IF was performed on pronase-digested, paraffin-embedded tissue (pronase IF).[8] Pronase IF for IgG, κ, and λ was also performed on FGN cases with apparent monotypic IgG staining on standard frozen tissue IF, as we have observed that some cases of FGN with light chain restriction on frozen tissue IF exhibit staining for both κ and λ on pronase IF.

Immunohistochemistry of DNAJB9

All instruments and reagents were purchased from Ventana Medical Systems, Inc. (Oro Valley, AZ) unless otherwise specified. DNAJB9 IHC was performed on 4-μm-thick, formalin-fixed, paraffin-embedded tissue sections mounted on charged slides. Tissue slides were dried and melted in an oven at 68°C for 20 minutes. Slides were stained with an anti-DNAJB9 rabbit polyclonal antibody (catalog no. HPA040967; 1/75 titer; Sigma-Aldrich, St. Louis, MO) on a Ventana BenchMark XT system. The staining protocol included online deparaffinization, heat-induced epitope retrieval (HIER) with Ventana Cell Conditioning 1 solution (CC1) for 32 minutes, and incubation with the primary antibody for 32 minutes at 37°C. Antigen–antibody reactions were visualized using Ventana OptiView Universal DAB Detection and OptiView Amplification Kits. Counterstaining was performed online using Ventana Hematoxylin II for 8 minutes, followed by bluing reagent for 4 minutes. Two renal pathologists (S.H.N. and M.P.A.) independently evaluated the stained tissues for DNAJB9 positivity without knowledge of the diagnoses. The Mayo Clinic Institutional Review Board approved this study, which was conducted in accordance with the Declaration of Helsinki.

Immunoelectron Microscopy

Immuno-EM studies were done on 8 renal biopsy samples from patients followed up at University Hospital of Poitiers (different from the 214 Mayo clinic cases), including 3 cases of FGN, 3 cases of AL-λ amyloidosis, and 2 cases of immunotactoid glomerulopathy. Immuno-EM for DNAJB9 was performed using an anti-DNAJB9 rabbit polyclonal antibody (Sigma-Aldrich, St. Louis,

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