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【 CASE REPORT 】

Extraglomerular Vascular Involvement of Glomerulopathy with Fibronectin Deposits

Masayuki Hara[1,2], Tetsuro Kusaba[1], Kenshi Ono[3], Naoko Masuzawa[4,5], Itaru Nakamura[1], Noriko Urata[1], Hirokazu Shiraishi[3], Shigeo Hara[6], Eiichi Konishi[4], Satoaki Matoba[3], Yayoi Shiotsu[1] and Keiichi Tamagaki[1]

Abstract:
Glomerulopathy with fibronectin deposits (GFND) is a rare hereditary kidney disease with autosomal dominant inheritance. A 21-year-old woman who had been diagnosed with GFND 10 years ago was admitted for investigation of a rapid decline in her renal function, hemolytic anemia, and cardiac dysfunction. A renal biopsy showed GFND accompanied by extraglomerular vascular lesions. Comprehensive treatments against hypertension and anemia improved the renal function. Although there have been few reports of vascular lesions in GFND, we suspect that endothelial hyperpermeability resulting from hypertension caused the fibronectin deposition and narrowing of the extraglomerular vascular lumens, thereby accelerating hypertension and inducing hemolytic anemia.

Key words: glomerulopathy with fibronectin deposits (GFND), vascular lesion, endothelial damage, hemolytic anemia, hypertension

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Introduction

Glomerulopathy with fibronectin deposits (GFND) is a rare hereditary kidney disease with autosomal dominant inheritance (OMIM #60894), first named fibronectin glomerulopathy in 1995 (1). GFND typically develops from approximately 10.5 to 30 years old, and end-stage renal disease occurs in 25% of patients (2). Castelletti et al. identified a mutation in the *FN1* gene encoding fibronectin at 2q32 as the gene responsible for GFND and reported that this mutation accounted for 40% of GFND cases (3).

Various renal histological findings, such as severe mesangial proliferative glomerulonephritis in a lobular form accompanied by extensive fibronectin deposition, are observed in GFND. However, previous reports have focused mainly on glomerular lesions, and little is known about extraglomerular involvement in GFND.

We herein report a young woman with GFND in whom renal biopsies were performed twice with a nine-year interval. Renal histology demonstrated extensive fibronectin deposition within not only the glomeruli but also the extraglomerular arterioles. The patient exhibited a rapid decline in her kidney function, which may be attributed to anemia, high blood pressure, and vascular lesions.

Case Report

A 21-year-old woman was admitted to our hospital for the investigation of hypertension and a rapid decline in her kidney function. At 11 years old, she had been diagnosed as GFND by renal biopsy. Her father also had renal failure, and hemodialysis had been initiated for him at 45 years old. A gene analysis of her and her father revealed a missense

[1]Department of Nephrology, Kyoto Prefectural University of Medicine, Japan, [2]Department of Nephrology, Omihachiman Community Medical Center, Japan, [3]Department of Cardiovascular Medicine, Kyoto Prefectural University of Medicine, Japan, [4]Department of Surgical Pathology, Kyoto Prefectural University of Medicine, Japan, [5]Department of Diagnostic Pathology, Otsu City Hospital, Japan and [6]Department of Diagnostic Pathology, Kobe City Medical Center, General Hospital, Japan
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Correspondence to Dr. Tetsuro Kusaba, kusaba@koto.kpu-m.ac.jp

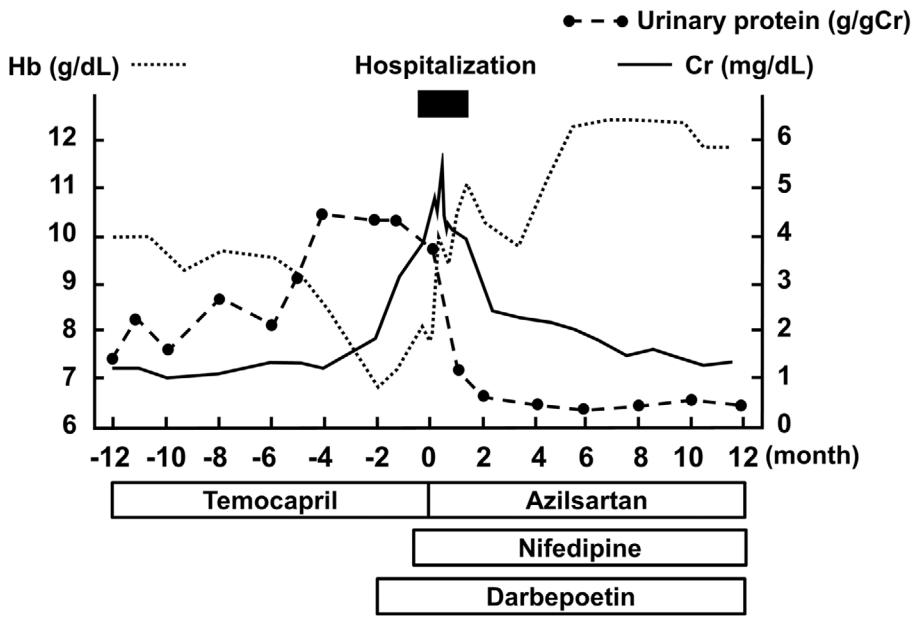

Figure 1. **Clinical course 12 months before and after hospitalization.**

mutation (exon19 c2915A>G, pY973c) in the *FN1* gene. A low dose of temocapril was administered to reduce proteinuria, and her blood pressure was maintained around 110/70 mmHg. Although her renal function and proteinuria had been stable for 10 years after the first biopsy (approximately serum creatinine=1.0 mg/dL), her serum creatinine increased rapidly during the 2 months before admission and was accompanied by moderate anemia and hypertension (Fig. 1).

On admission, her blood pressure was 160/110 mmHg, and a laboratory analysis revealed renal dysfunction, anemia, hematuria, and proteinuria (Table), and all of these parameters were notably worse than they had been at 2 months prior to admission. In addition, a mild increase in lactate dehydrogenase and decreased haptoglobin were noted, suggesting hemolytic anemia. There were no newly identified serological abnormalities suggesting autoimmune diseases or infectious diseases. Chest X-ray demonstrated cardiomegaly accompanied by pleural effusion, and echocardiography revealed a moderately decreased systolic function.

In order to clarify the cause of the rapid worsening of the renal function, we performed a renal biopsy again. At the previous renal biopsy performed 10 years earlier, light microscopy had shown enlargement of the glomeruli with markedly increased mesangial extracellular periodic acid-Schiff (PAS)-positive material and a lobular glomerular appearance (Fig. 2A). Mild arteriolar PAS-positive deposition and extraglomerular neovascularization around the vascular pole of the glomerulus had also been observed. At the second renal biopsy, light microscopy revealed extensive glomerular lesions (Fig. 2B) and progression of interstitial fibrosis (20% at the first to 60% at the second biopsy, approximately). In particular, the extraglomerular vascular lesions had significantly worsened, and there were two different pathological changes in vascular walls: PAS-positive deposition in the subendothelial spaces in arterioles (Fig. 2C) and mucoidal intimal edema (Fig. 2C, D), which is frequently observed in malignant nephrosclerosis (4). These vascular lesions resulted in severe narrowing of the vascular lumen in not only the arterioles but also the small arteries (Fig. 2C, D). Immunofluorescence staining was negative for immunoglobulins and complements within the glomerulus, but IgM and C3 were positive in the extraglomerular vasculature (Fig. 2E, F). Electron microscopy of the glomerulus revealed substantial electron-dense deposition in the subendothelial and mesangial spaces (Fig. 2G). Furthermore, electron microscopy of the small arteries revealed massive electron-dense deposition in the subendothelial spaces, resulting in the occlusion of the vascular lumen (Fig. 2H).

To determine the origin of deposited fibronectin, we performed immunostaining using an antibody against both soluble and insoluble forms of fibronectin (IST-4) and an antibody against insoluble forms of fibronectin (IST-9) (5). IST-9 immunostaining was negative in the glomeruli and vasculatures (Fig. 2I), whereas IST-4 immunostaining was positive in the mesangium and mesangial nodules within glomeruli collected at the first and second renal biopsies (Fig. 2J). IST-4 immunostaining was also positive in the vascular wall of small arteries and arterioles (Fig. 2K). Given the undetermined cause of heart failure, we also performed a biopsy of the myocardium. A pathological analysis revealed no specific histological changes, and an immunostaining analysis using IST-4 and IST-9 showed no soluble form of fibronectin deposition in the cardiac tissue (Fig. 2L).

Based on the findings of both serial renal biopsies, a diagnosis of GFND accompanied by extraglomerular vascular involvement was made. This condition may have been responsible for the rapid decline in the renal function and microangiopathic hemolytic anemia. Intensive antihypertensive treatment using a combination of azilsartan and nifedipine

Table. **Laboratory Data on Admission.**

Hematology		**Serology**	
WBC	9,000 /μL	IgG	702 mg/dL
Hb	8.2 g/dL	IgA	149 mg/dL
MCV	89.9	IgM	121 mg/dL
Platelet	20.5×10^4 /μL	Complement3	90.3 mg/dL
		Complement4	31.7 mg/dL
Biochemistry		CH50	62.8 U/mL
Total protein	5.7 g/dL	C-reactive protein	0.23 mg/dL
Albumin	3.3 g/dL	ANA	<×40
Urea nitrogen	46.6 mg/dL	a-beta2GPI	<8 U/mL
Creatinine	4.6 mg/dL	LA	3.9 s
eGFR	11.3 mL/min/1.73 m^2	Direct Coombs	-
Sodium	140 mEq/L	Indirect Coombs	-
Potassium	4.4 mEq/L	HBs-Ag	-
Chloride	106 mEq/L	HCV-Ab	-
Calcium	8.9 mg/dL	Cryoglobulin	-
Phosphorus	5.8 mg/dL	PRA	34.8 ng/mL/h
Total bilirubin	0.49 mg/dL	PAC	284 pg/mL
AST	21 IU/L	**Urinalysis**	
ALT	14 IU/L	sp gr	1.008
LDH	476 IU/L	pH	6.0
LDL cholesterol	132 mg/dL	Protein	1.89 g/gCreatinine
HDL cholesterol	51 mg/dL	Glucose	-
TG	147 mg/dL	RBC sediment	1-4 /high power Field
Hemoglobin A1c	4.5 %	Beta-2-MG	12,634 μg/gCreatinine
Haptoglobin	5 mg/dL	NAG	17.9 U/gCreatinine

eGFR: estimated glomerular filtration rate, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, CH50: 50% hemolytic unit of complement, ANA: antinuclear antibody, a-beta2GPI: anti-beta2-glycoprotein I antibody, LA: lupus anticoagulant, HBs-Ag: hepatitis B surface antigen, HCV-Ab: hepatitis C virus antibody, PRA: plasma renin activity, PAC: plasma aldosterone concentration, sp gr: specific gravity, RBC sediment: red blood cell sediment, beta-2-MG: beta-2-microglobulin, NAG: N-acetyl-beta-D-glucosaminidase

and appropriate control of hemoglobin levels by an erythropoiesis-stimulating agent (ESA) dramatically improved the patient's cardiac and renal phenotypes (serum creatinine decreased to 1.5 mg/dL and proteinuria decreased to 0.3 g/gCr), and her condition has remained stable for several years.

Discussion

Fibronectin is a large dimeric glycoprotein that is involved in cellular adhesion, migration, and proliferation (6). There are two fibronectin isoforms: an insoluble form derived from local matrix-producing cells and deposited in the extracellular matrix and basement membranes (cellular form), and a soluble form derived from hepatocytes that circulates in the blood (plasma form) (3). Considering the rapid recurrence of GFND in transplanted allografts (7) and immunobinding of the specific antibody IST-4 (1), deposition of the soluble form of fibronectin is considered to be responsible for GFND development. The soluble form of fibronectin assembles into organized fibrils in the extracellular matrix via complex fibronectin-fibronectin and fibronectin-cell surface proteoglycan interactions (3).

The mutation of the *FN1* gene in our patient is common among GFND patients and located at the heparin-binding domain of fibronectin (8). Fibronectin formed by the mutant gene in our patient has an impaired heparin-binding domain, which reduces its ability to assemble, leading to excess fibronectin that is eventually deposited in the extracellular matrix (3, 9).

The most striking histological findings in our patient were the detection of fibronectin deposition in not only the glomeruli but also the extraglomerular vasculature of the kidneys. Electron microscopy revealed notable subendothelial deposits in arterioles, resulting in severely narrowed vascular lumens. IST-4 and IST-9 immunostaining in renal arterioles indicated that the deposits originated from the soluble form of fibronectin, just as seen in glomeruli. In addition, IST-4 immunostaining was negative in the small arteries of the heart, indicating that fibronectin deposition seemed to specifically occur within the renal small vessels and not within the systemic vasculature.

Extraglomerular neovascularization around the vascular pole of the glomerulus was another notable histological finding in our patient. This structure is frequently observed in diabetic nephropathy and represents a shortcut between af-

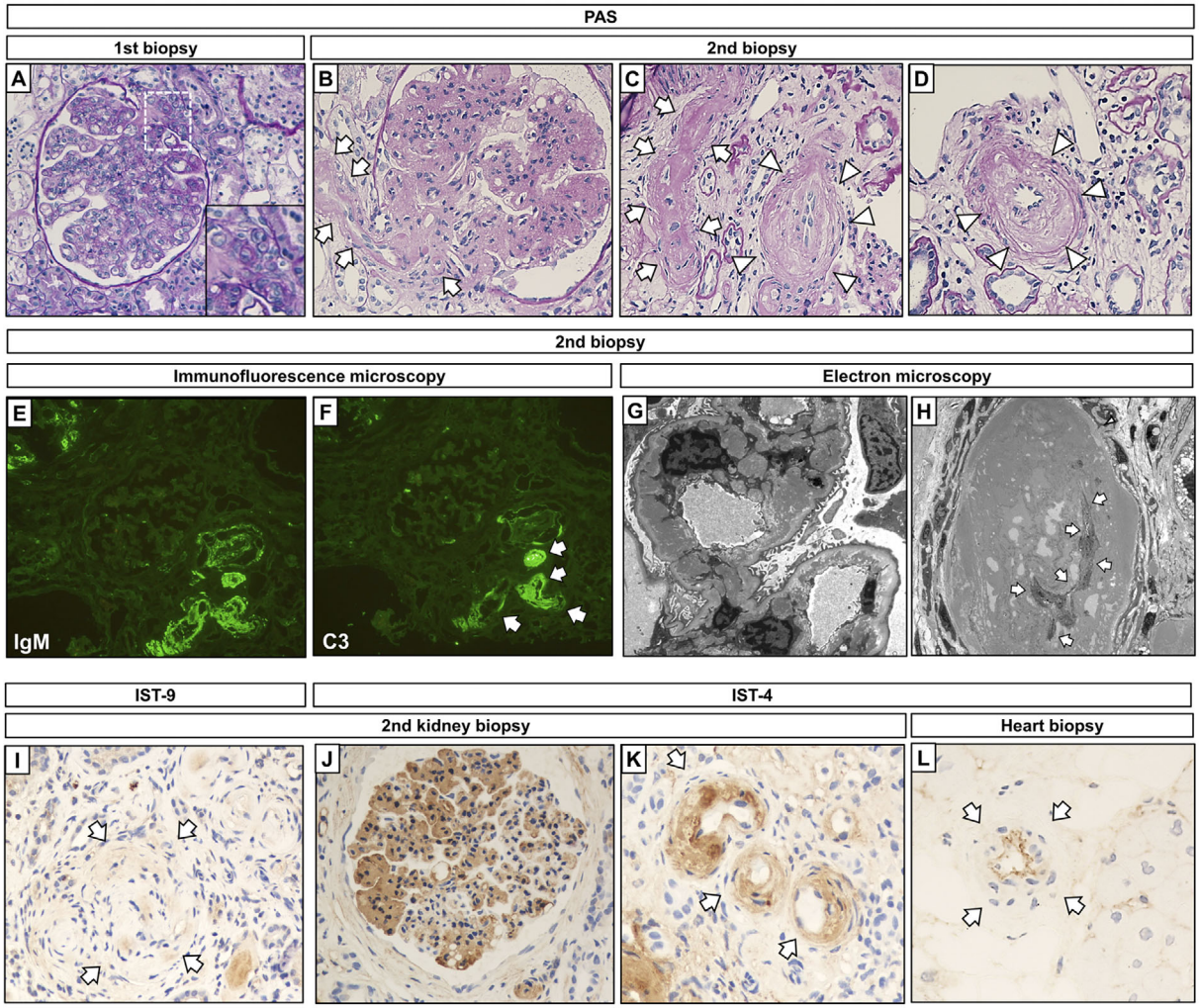

Figure 2. **Renal histology. (A) Light microscopic findings of periodic acid-Schiff (PAS) staining of the first biopsy. Diffuse mesangial proliferation accompanied by neovascularization of the vascular pole of the glomerulus (small square). (B-D) PAS staining of the second biopsy. (B) Diffuse mesangial proliferation and nodular lesions in the glomerulus accompanied by PAS-positive deposition of arterioles (arrows). (C) Substantial PAS-positive deposition of arterioles (arrows) and occlusion of small arteries by mucinous intimal thickening (arrowheads). (D) Stenosis of small arteries by thickening of intimas with mucoid matrices (arrowheads). (E, F) Immunofluorescence staining. Positive staining of C3 (E) and IgM (F) in extraglomerular vasculatures (arrows) but not within the glomerulus. (G, H) Electron microscopy of the glomerulus (G) and extraglomerular arterioles (H). (G) Massive electron-dense deposition at subendothelial and mesangial spaces. (H) Expansion of the subendothelial space by electron-dense deposition, resulting in the occlusion of arteriolar lumens. (I) Negative immunostaining of IST-9 (recognizing only the insoluble form of fibronectin) in arterioles (arrows) in the kidneys. (J, K) Substantial positive immunostaining of IST-4 (recognizing both the soluble and insoluble forms of fibronectin) within the glomerulus (J) and subendothelial spaces of renal arterioles (arrows in K) of the second kidney biopsy, but negative staining in cardiac arterioles (arrows in L). Magnification ×400 in (A-F, I-L), ×5,000 in (G) and ×2,500 in (H).**

ferent arterioles and peritubular capillaries (10-12). In diabetic nephropathy, the occlusion of glomerular capillaries leads to glomerular hypertension in the remnant glomeruli. Glomerular hypertension and elevated vascular endothelial growth factor in diabetes drive the progression of neovascularization (13). In GFND, as massive fibronectin deposition in the subendothelial space of glomeruli narrows glomerular capillaries (14), we suspected that glomerular hypertension and subsequent neovascularization around the vascular pole of the glomerulus had developed in our patient through the same mechanisms as seen in diabetic nephropathy.

We also observed another type of renal vascular injury: severely thickened intimas with mucoid matrices, which is a pathological feature of malignant nephrosclerosis (4). Given these renal vascular lesions, the deposition of the soluble form of fibronectin in the renal vasculatures of our patient

may have developed via the following mechanism: The positive staining of C3 and IgM in the renal arterioles and negative staining of these in the glomeruli suggest that different mechanisms underly the fibronectin deposition in glomeruli and vessels. The IgM and C3 depositions in the vascular walls may have been "non-specific" and caused by hyperpermeability of endothelial cells, which is found in hypertensive patients, rather than a specific immune reaction (4). In addition, the occlusion of glomerular capillaries by the aforementioned mechanisms may have increased the arterial pressure in the afferent arterioles, thereby exacerbating the endothelial damage. Furthermore, unlike the other organs, including the heart, the unique anatomical structure of the renal vasculature, including the glomerulus and afferent arterioles, may have been involved in this kidney-specific phenomenon. Therefore, the deposition of the soluble form of fibronectin as well as IgM and C3 specifically within the renal vasculature was attributed to the extensive endothelial hyperpermeability as a consequence of a vicious cycle of severe hypertension, occlusion of glomerular capillaries, and pressure-induced endothelial damage in the preglomerular vasculatures.

In contrast to previously reported GFND cases, our patient exhibited hemolytic anemia, severe hypertension, and cardiac dysfunction, which may have contributed to the rapid decline in the renal function. The etiology of these clinical features was not identified, but we suspect that narrowed renal arterioles induced the activation of the renin-angiotensin system (RAS) and led to severe hypertension. In addition, hypertension induced endothelial damage and the exacerbation of narrowing arterioles, eventually resulting in intravascular hemolytic anemia. At present, there is no specific treatment for GFND, and RAS inhibitors have been used to reduce proteinuria (15). Comprehensive treatments against hypertension using RAS inhibitors and against anemia using ESA ameliorated the aforementioned vicious cycle and thereby improved the renal function in the present patient.

The authors state that they have no Conflict of Interest (COI).

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