in an available sample (Fig. 3B). Tannic acid staining of III-1's first and second biopsies revealed fibrillar collagen material in the GBM by electron microscopy. Immunofluorescence analysis showed focal staining of type III collagen in glomeruli (Fig. 4), indicating that tannic acid staining or immunofluorescence analysis is more sensitive for detecting fibrillar deposition in GBM, than the detection of changes in electron-lucent area using electron microscopy. As podocin expression is regulated by LMX1B[6, 19-23], we examined whether changes in podocin expression could be observed in early stage of disease progression. While linear expression of podocin is observed in control samples, expression significantly decreased in samples, even at two years of age (III-1) (Fig. 5). This change was also observed in proband's second renal biopsy.

Discussion

Using WES, we identified heterozygous *LMX1B* R246Q mutation in a large family with nonsyndromic autosomal dominant nephropathy. As skeletal and nail anomalies were not observed and electron microscopy did not indicate a moth-eaten GBM appearance unique to NPS nephropathy, clinical findings and routine renal histology did not provide diagnostic suspicion of *LMX1B* mutation. Genetic investigations utilising next-generation sequencing technologies are useful in diagnosing familial nephropathy

with uncertain cause. *LMX1B* is a strong candidate gene, which is capable of causing autosomal dominant renal-limited disease.

To date, *LMX1B* R246 heterozygous mutation (R246Q or R246P) has only been reported in two papers. Boyer *et al.* described three large families exhibiting *LMX1B* mutation without renal manifestation; however, details and longitudinal clinical course of disease were unclear.[7] Isojima *et al.* reported on an individual with isolated nephropathy who had been tested for *LMX1B* mutation because of characteristic pathological findings such as moth-eaten appearance of GBM.[6] The present report is the first to describe the long-term clinical course of proteinuria and renal function in a family with *LMX1B* R246Q.

Clinical observation for more than ten years demonstrated that five out of six affected patients in our family exhibited proteinuria without oedema during early childhood. Chronological assessment of urinary abnormality or kidney function revealed proteinuria gradually increased to nephrotic levels in their adolescence. In all affected patients, normal renal function was maintained throughout childhood; however, renal function gradually decreased and progressed to ESRD in adulthood (Fig. 3). The previously reported isolated case also maintained normal renal function at the age of nine.[6] Furthermore, two out of twelve patients in Boyer's report were also diagnosed

during their schoolage.[7] These findings suggest a large fraction of patients with R246Q mutation may develop asymptomatic proteinuria or haematoproteinuria during early childhood. Through childhood urinary screening, early detection before renal function deterioration could be possible, at least in a proportion of patients carrying the R246Q mutation. Boyer *et al.* also reported that eight out of twelve patients with R246Q mutation progressed to chronic kidney disease (CKD) stage II–IV. Among them, five individuals had reached ESRD.[7] Together with the present report, all patients with R246Q in middle age or above exhibited decreased renal function. Interestingly, previous studies estimated renal failure occurs in only about 5–10% of patients with typical NPS,[1-3, 5] with severity of nephropathy being extremely variable both within and between families.[2] Therefore, renal prognosis for individuals with the R246Q mutation may be poor compared with typical NPS, and progression to CKD may be inevitable without treatment.

Persistent severe proteinuria often leads to loss of kidney function. Despite the genetic causes, use of ACEI has been demonstrated to be effective at delaying disease progression in a variety of proteinuric glomerular diseases. For example, early diagnosis and renoprotective therapy with ACEI in oligosymptomatic patients with Alport syndrome can delay onset of ESRD.[24] These reports raise the possibility that treatment

with ACEI might provide renoprotection in patients with LMX1B mutation. Indeed, Lemley *et al.* report effectiveness of ACEI and/or ARB usage in NPS nephropathy.[5] Dramatic antiproteinuric effects in infantile nephrotic syndrome caused by *LMX1B* mutation have also been demonstrated.[4] In the present report, three individuals (II-4, III-1 and III-3) have been treated with renin-angiotensin-aldosterone system (RAAS) inhibitors, such as ACEI and ARB. While one adult patient (II-4) has already been diagnosed as CKD stage II, the other two patients (still in their teens) maintained renal function. Thus, a longitudinal study following a large number of patients is needed to clarify protective effects of RAAS inhibitors on renal prognosis of R246Q mutation.

In our investigation, kidney histology of two childhood patients demonstrated MCD, while that of an adult patient exhibited FSGS (Table 2 and Fig. 3). In previous reports, several patterns of pathology (such as MCD, FSGS and mesangial proliferative glomerulonephritis) were observed in patients manifesting renal-limited phenotypes of *LMX1B* mutation.[6, 7, 25] In this manner, light microscopy findings for this disease varied by disease duration. Furthermore, irregular thickening of GBM with electron-lucent areas were not always detected by electron microscopic examination.[6, 7, 25] Interestingly, despite a lack of moth-eaten appearance in our case, collagen fibrils within the GBM were detected by tannic acid staining using electron microscopy and type III collagen

was focally stained in glomeruli, as detected by immunofluorescence microscopy. These results indicate that tannic acid staining or immunofluorescence analysis may be more sensitive for detecting fibrillar deposition in GBM than change in electron-lucent areas measured by electron microscopy. Therefore, collagen staining by electron and/or immunofluorescence microscopy may facilitate diagnosis of *LMX1B*-associated nephropathy, especially in patients with autosomal dominant nephropathy with uncertain cause.

Studies utilising Lmx1b-null mice have demonstrated possible downstream targets of LMX1B include α3 and α4 type IV collagens, podocin and CD2AP in kidney.[6, 19-21, 26] *In vitro* studies also demonstrated that podocin expression is regulated combinatorially by Lmx1B and FoxC.[23] However, at least in some typical NPS patients with a thickened GBM containing collagen type III deposits, podocin protein expression was not altered, indicating the possibility that decreased podocin expression is secondary to podocyte injury, not the primary effect of LMX1B on its transcription.[22] Notably, in our patient with R246Q mutation, podocin expression was significantly decreased compared with MCD from the first renal biopsy taken at two years of age. Whether or not this change is a general feature of R246Q patients remains unclear, though, this case suggests that R246Q mutation affects podocyte slit diaphragm integrity, even in early stages of

disease progression.

However, mechanisms by which R246 mutation causes renal-limited phenotypes remain unexplained. Boyer *et al.* suggested arginine 246 might be critical for stabilizing *LMX1B* homeodomain/DNA interactions.[7] Further, Isojima *et al.* speculated that phenotypic differences between typical NPS and renal-limited phenotypes might result from residual LMX1B transcriptional activity using *in vitro* experiments.[6]

In conclusion, genetic analyses were helpful to diagnose LMX1B-associated nephropathy in autosomal dominant renal-limited disease, even in cases in which a moth-eaten appearance was not confirmed by electron microscopy. Clinical manifestation can vary, but preforming urinary screening examinations to detect early changes may be possible; consequently, renoprotective treatments may be administered early to delay disease progression. Furthermore, R246Q mutation results in subtle but distinct podocyte damage in early stages and manifests as various types of histological alterations in adulthood that finally result in renal dysfunction.

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Conflict of interest: none

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Table 1. Clinical findings of *LMX1B* R246Q mutation

Patient	Age at Onset (yr)	Extra Renal Feature	At onset			At Last Follow-up or At induction of HD			
			Proteinuria Dipstick or g/g Cr	Hematuria Dipstick	edema	Proteinuria (g/g Cr)	Hematuria Dipstick	Albumin (g/dL)	eGFR (ml/min/1.73m^2) and Age (yr)
Ⅰ-1	Unknown	-	ND	ND	ND	ND	ND	ND	ESRD at 40
Ⅱ-2	6	-	+++	+	-	9.5	+	3.43	ESRD at 38
Ⅱ-4	13	-	++	-	-	4.5	+	2.95	62.2 at 42
Ⅲ-1	1	-	+++	+++	-	3.7	++	2.78	187.6 at 18
Ⅲ-2	11	-	++	-	-	0.8	+	2.99	154.9 at 15
Ⅲ-3	1	-	1.6	+	-	1.5	++	2.40	161.1 at 12

ND, not determined; HD, hemodialysis; ESRD, end stage renal disease

Table 2. Kidney histology of *LMX1B* R246Q mutation

Patient	Age at Renal biopsy (yr)	Kidney Histology		
		LM	IF	EM
Ⅱ-2	15	MCD without TI change	No deposition	ND
Ⅱ-4	39	FSGS with focal interstitial fibrosis and tubular atrophy	No deposition	Focal foot process effacement Moth-eaten appearance (-)
Ⅲ-1 (1st)	2	MCD without TI change	No deposition	Focal foot process effacement Focal thinning of GBM Moth-eaten appearance (-)
Ⅲ-1 (2nd)	10	MCD without TI change	No deposition	Focal foot process effacement Focal thinning of GBM Moth-eaten appearance (-)

LM, light microscopy; IF, Immunofluorescence microscopy; EM; electron microscopy; MCD, minimal change disease; FSGS, focal segmental glomerulosclerosis; TI change, tubulo-interstitial change; GBM, glomerular basement membrane; ND, not determined

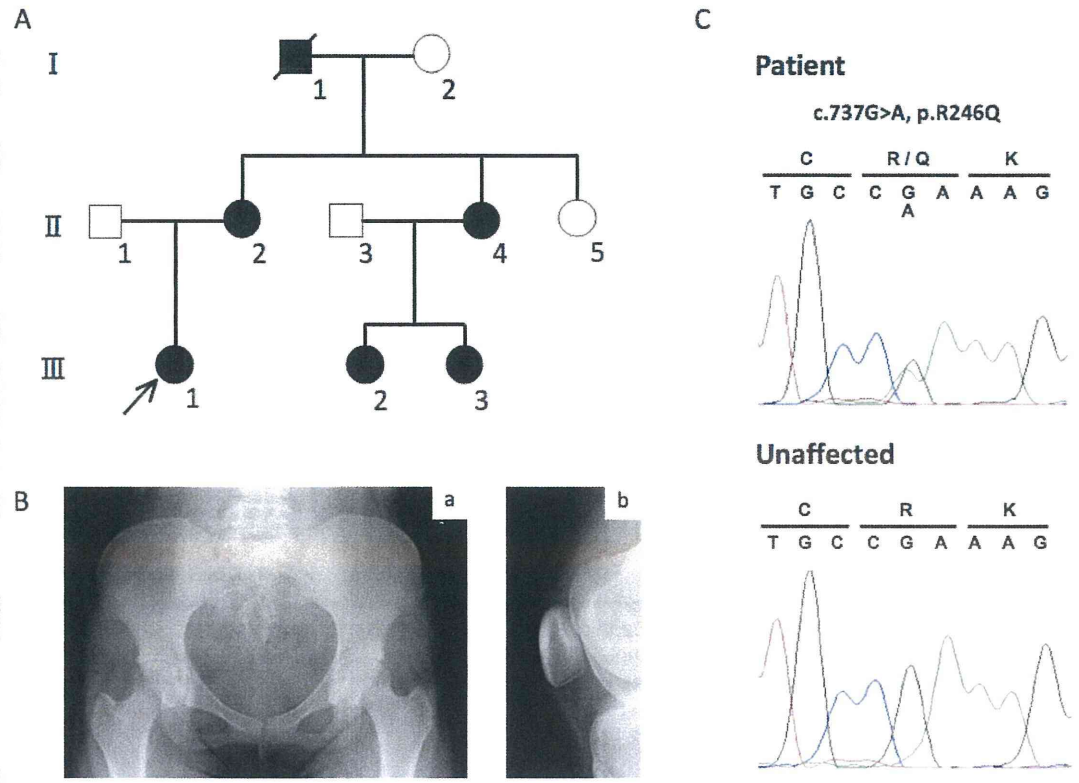

Figure 1: Pedigree and *LMX1B* mutation. (A) Pedigree demonstrated an autosomal dominant pattern of disease, and included six affected patients over three generations. DNA is available from five patients (II-2, II-4, III-1, III-2 and III-3) and two unaffected relatives (II-1 and II-3). WES was performed in these seven persons. (B) Radiographs of the pelvis (a) and knee (b) of proband (III-3). No iliac horn and patella dysplasia can be noticed. (C) *LMX1B* heterozygous G-to-A mutation in exon 4 (c.737G>A, p.R246Q) was confirmed by Sanger sequencing in all affected patients.

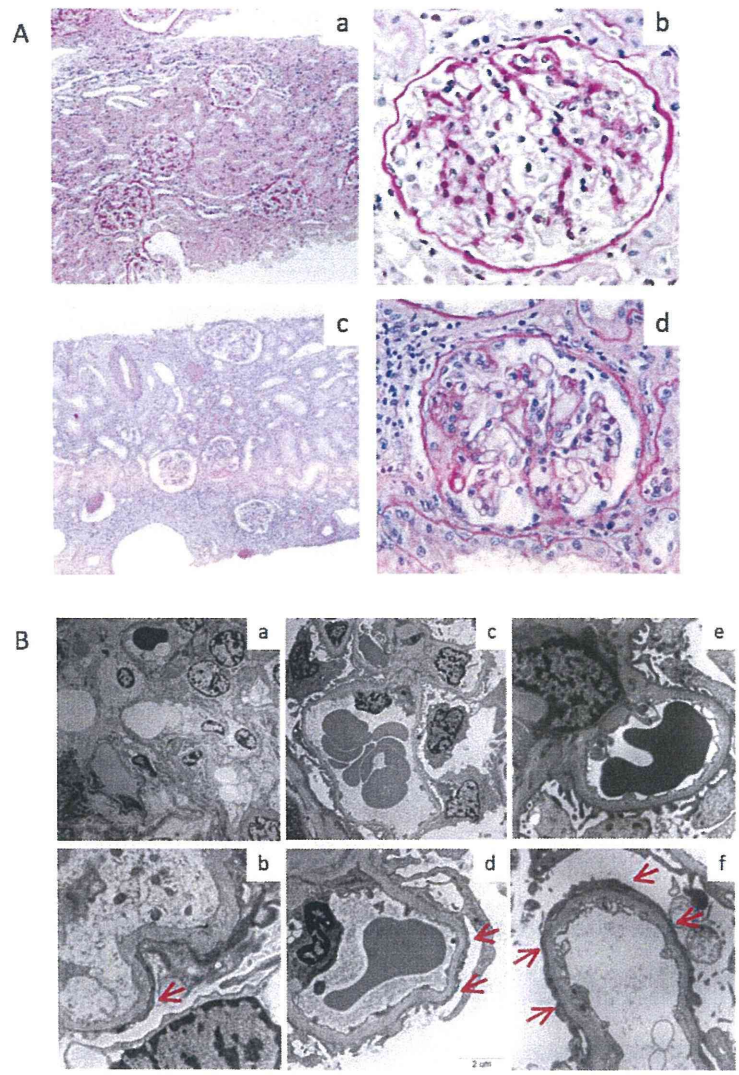

Figure 3: Kidney histology of child and adult patients. (A) Light microscopy of proband's (III-1) second biopsy and her aunt's (II-4) biopsy (Periodic acid-Schiff stain). Patients III-1 (a and b) and II-4 (c and d) show a normal glomerulus and focal segmental glomerulosclerosis, respectively. (B) Electron microscopy of proband's (III-1) and her aunt's (II-4), (a) and (b) are first, (c) and (d) are second biopsy of III-1, and (e) and (f) are biopsy of II-4. Focal foot process effacement is noticed in both patients (red arrows). Focal thinning of GBM is observed on patient III-1, the GBM diameter of the first (a) and second biopsy (c) is 160 nm and 180 nm, respectively. The GBM of patient II-4 is normal (e). Electron lucent areas in the GBM, referred to as a moth-eaten appearance, and most specific histological changes of NPS are not observed. Electron dense deposits are not present.

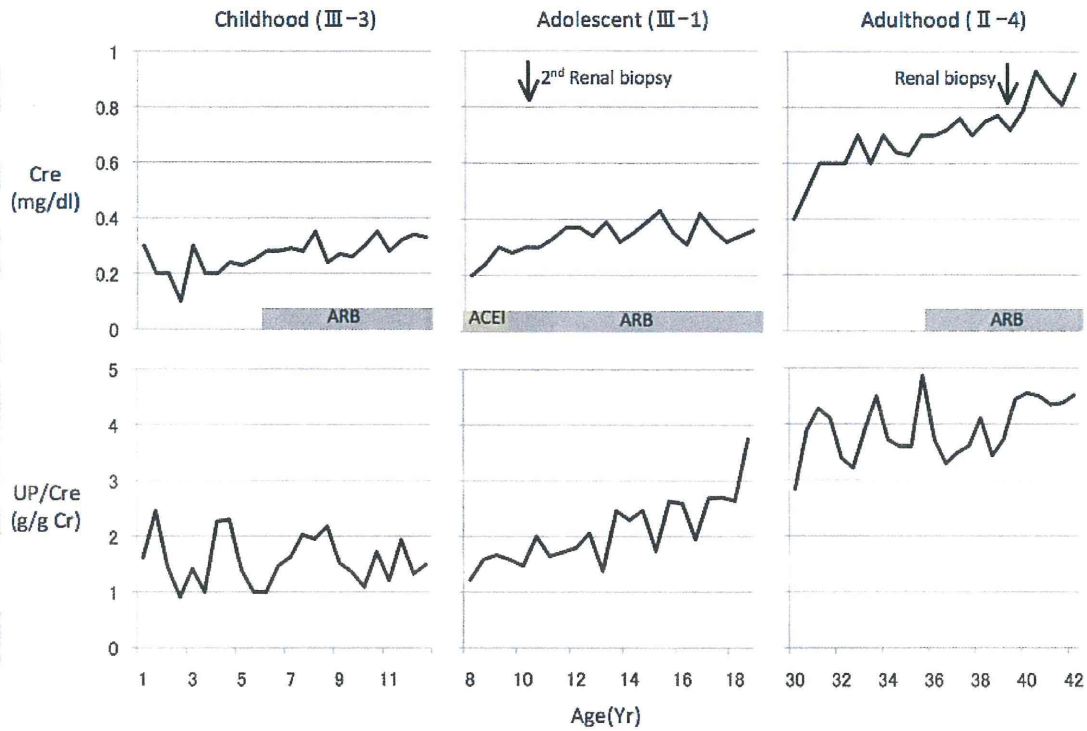

Figure 2: Progression of proteinuria and renal function. Urinary protein/creatinine ratio and serum creatinine level of patient (III-3, III-1 and II-4) were presented. Proteinuria was recognised in early childhood (III-3). Proteinuria gradually increases to nephrotic levels in their adolescence, and normal renal function is maintained in child patients (III-3 and III-1). Renal function gradually decreased and progressed in adult patients (II-4).

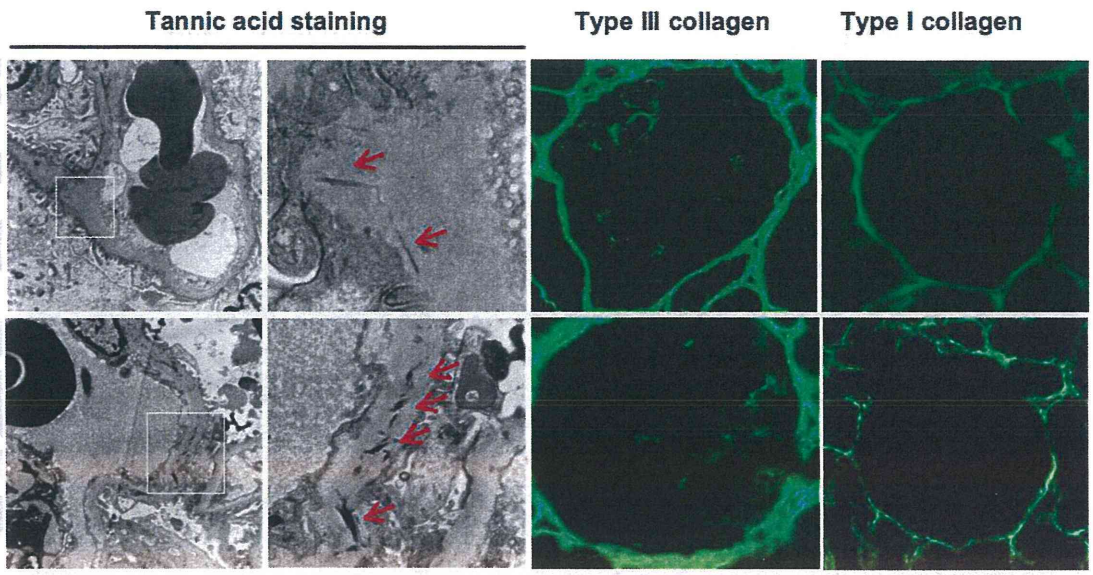

Figure 4: Collagen staining of the proband (III-1). Upper panel shows first biopsy specimens taken at an age of 2 years. Lower panel shows second biopsy specimens from 10 years of age. Collagen staining with tannic acid in electron microscopic examination indicates collagen fibrils in GBM (red arrows). Second lane is enlarged image of collagen fibrils. Type III collagen is focally expressed in glomeruli at both of first and second biopsy, however type I collagen is not observed.

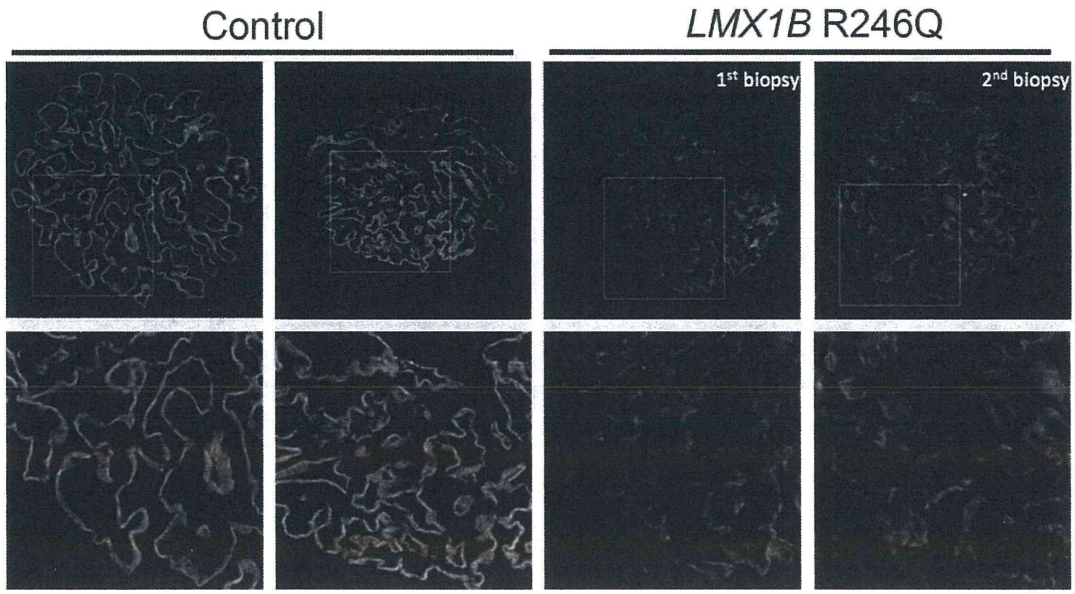

Figure 5: Immunohistological analysis of podocin in glomeruli from the patient (III-1). Renal biopsy specimen of MCD patients is used as an age matched control of proteinuric kidney disease. Lower panel is enlarged image. Podocin expression significantly decreases compared with control even in first biopsy samples at 2 years of age. Podocin expressions from the second biopsy are identical to those from the first.

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CASE REPORT

Glomerulopathy with distinctive fibrillar deposits but lacking glomerular deposition of type III collagen

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Abstract A 62-year-old woman with nephrotic syndrome underwent a renal biopsy. Under light microscopy, the biopsy findings included lobulation and enlargement of glomeruli, occasional thickening of glomerular capillary walls, and narrowing of the capillary lumen by swollen endothelial cells. Congo red staining was negative for amyloid. No significant intraglomerular fibrin deposition was found by phosphotungstic acid hematoxylin staining. Immunofluorescence microscopy showed no deposition of immunoglobulin G, A, or M; no κ or λ light chains; and no C3 or C1q. Electron microscopy revealed distinctive subendothelial and mesangial fibrillar deposits, mesangial cell interposition, and swelling and vacuolization of endothelial cells resulting in capillary lumen narrowing. Although some curvilinear fibrillar deposits mimicked the bundles of type III collagen fibers seen in collagenofibrotic glomerulopathy, neither glomerular deposition of type III collagen nor elevation of serum procollagen III peptide was noted. This glomerulopathy does not fulfill any known disease entities with non-amyloid non-immunoglobulin-derived organized glomerular deposits.

Keywords Renal disease with organized deposits · Collagenofibrotic glomerulopathy · Type III collagen

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Introduction

In this report, we present the case of a 62-year-old woman with nephrotic syndrome, whose renal biopsy was significant for subendothelial and mesangial fibrillar electron-dense deposits. Although some curvilinear fibrillar deposits mimicked the bundles of type III collagen fibers seen in collagenofibrotic glomerulopathy and nail–patella syndrome, neither glomerular deposition of type III collagen nor elevation of serum procollagen III peptide was noted.

Case report

A 62-year-old Japanese woman was admitted to our hospital for treatment of edema. She had a 14-year history of hypertension and had been treated to maintain a blood pressure of approximately 150/80 mmHg at another clinic. Proteinuria had developed 12 years before admission to our hospital, but it had been followed without treatment. She had no history of alcoholism, exposure to chemicals, or drug abuse, but she had a smoking history of 10 cigarettes per day for 42 years (21 pack-years). There was no family history of renal disease. Physical examination showed periorbital and pretibial pitting edema, but there were no

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