Pediatr Nephrol (2001) 16:482–487

CLINICAL NEPHROLOGY / ORIGINAL ARTICLE

Masaru Tada · Shiro Jimi · Satoshi Hisano
Yoshie Sasatomi · Kazuhiro Oshima
Hirofumi Matsuoka · Shigeo Takebayashi

Histopathological evidence of poor prognosis in patients with vesicoureteral reflux

Received: 3 January 2000 / Revised: 18 January 2001 / Accepted: 18 January 2001

Abstract Patients with vesicoureteral reflux (VUR) often develop reflux nephropathy with focal segmental glomerular sclerosis (FSGS), although the exact mechanisms leading to the development of this complication are unknown. To determine the early changes in glomeruli of VUR patients that ultimately cause poor renal outcome, we examined morphometrically renal biopsies of 16 young patients (age 10–20 years) with VUR at baseline pre-operatively. Patients were divided into two groups, those who subsequently showed good prognosis and those with poor renal prognosis at the end of a 10-year follow-up period. Patients with poor prognosis had worse proteinuria and lower creatinine at baseline than those with good prognosis. We also examined 40 age-matched control cases with previous temporal microhematuria and/or proteinuria but normal renal function and histology. Although the mean diameter of glomerular capillary did not change in VUR cases irrespective of prognosis, glomerular capillary length increased by 125% in cases with good prognosis, and 335% in cases with poor prognosis ($P<0.01$). Cystically expanded capillaries, with diameter ≥95% of that in age-matched control, were detected in five of eight patients with poor prognosis, but only in one of eight patients with good prognosis. In VUR, the number of podocytes/capillary diminished with increased length of the capillaries. Tuft adhesion to Bowman's capsule and podocyte detachment were primarily found in patients with poor prognosis. Our results suggest that lengthening of glomerular capillaries in young patients with VUR is a compensatory reaction to hyperfiltration. The appearance of cystic capillary expansion, podocyte detachment and/or tuft adhesion to Bowman's capsule in such glomeruli may be important indicators of renal prognosis in patients with VUR. These changes may lead to FSGS due to podocyte injury in patients with VUR, with subsequent deterioration of renal function.

Keywords Vesicoureteral reflux · Reflux nephropathy · Capillary elongation · Adhesion · Sclerosis

M. Tada · S. Jimi · S. Hisano · Y. Sasatomi · S. Takebayashi (✉)
The Second Department of Pathology, School of Medicine,
Fukuoka University, 7-45-1 Nanakuma, Jonan-ku,
Fukuoka 814-0180, Japan
e-mail: take@fukuoka-u.ac.jp
Tel.: +81-92-8011011, ext. 3285, Fax: +81-92-8638383

K. Oshima · H. Matsuoka
Department of Urology, School of Medicine, Fukuoka University,
7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

Introduction

Vesicoureteral reflux (VUR) in children is due to an inherited defect of the ureter or vesicoureteric valve. The condition is often associated with various complications such as urinary tract infection [1,2], which result in renal tissue damage including nephron scarring [3], collectively known as reflux nephropathy (RN) [1, 2]. Most patients with RN show complete recovery after corrective surgery [2], although some develop irreversible deterioration of renal function associated with poor prognosis [4]. While the clinicopathological changes in such patients have been well characterized, the early pathological changes in the glomeruli of patients with VUR are still unclear.

Previous studies have described two types of glomerular changes in non-scarring renal regions of patients with RN. These include glomerular hypertrophy [4, 5, 6, 7,8] and focal glomerular sclerosis [4, 6,8]. The former is more commonly seen and is due to glomerular hyperfiltration, while the latter is found in patients with advanced renal pathology [2]. Many previous studies using animal models of hyperfiltration [9, 10, 11, 12] identified the possible causes of deterioration of renal function; however, such mechanisms have not yet been confirmed in humans.

In the present study, we have characterized the early morphological changes in glomeruli in a group of young patients with VUR and retrospectively followed up these patients to determine the clinicopathological outcome. Our results showed that cystic dilatation of glomerular

capillary induced podocyte injury and detachment, which strongly influenced renal prognosis in patients with VUR.

Materials and methods

Patients

Sixteen patients (eight males and eight females) with congenital bilateral VUR of severity exceeding grade III, according to the International Classification for VUR [13], as diagnosed radiologically using a contrast media and cortical scarring by ^{99m}Tc-DMSA scintigram, were enrolled in the present study. These patients represented all those admitted to the Department of Urology at Fukuoka University Hospital, Fukuoka, Japan, between 1985 and 1995. Open kidney biopsy was performed in all patients just before cross-trigonal ureteroneocystostomy for VUR. Age at biopsy was 14.1±3.4 years (mean±SD, range, 10–20 years). Patients were followed for 10 years after the operation and urinary protein, serum creatinine and creatinine clearance were measured just before the operation (baseline) and at several time periods throughout the study. Patients were examined retrospectively after the 10-year follow-up period. Creatinine clearance of <60 l/day during the study indicated deterioration of renal function. Accordingly, patients were designated into those with good prognosis and patients with poor prognosis (Table 1). Eight cases showed good prognosis (age 13.7±3.5 years at first biopsy, five males and three females), while eight patients showed poor prognosis (age 14.8±3.3 years at biopsy, two males and six females). Age-matched control patients (age 14.8±2.2 years at biopsy, 19 males and 21 females) were also included in the study. The latter group consisted of patients with a history of microhematuria, but showed no serum abnormalities and had normal blood pressure, and normal renal function and normal glomerular, tubular, interstitial and vascular structures by light and electron microscopy and immunohistological examination for immune complexes and fibulin.

Fig. 1a–c Representative glomeruli of VUR and control subjects. **a** Control (male, 17 years), **b** VUR with good prognosis (male, 16 years), **c** VUR with poor prognosis (male, 18 years). Original magnification: ×300

Histopathological examination

Kidney biopsy specimens were routinely examined by light and electron microscopy and immunofluorescence staining. For light microscopy, paraffin-embedded sections were stained with hematoxylin-eosin, periodic acid-Schiff and periodic acid-methenamine silver (PAM) staining. Frozen sections were used for direct immunofluorescence staining using antisera for immunoglobulins (IgG, IgM, IgA), complements (C3, C1q) and fibrinogen. For electron microscopy, biopsy samples were fixed with 1.4% glutaraldehyde, post-fixed with osmium tetroxide, and embedded in Epon resin. Ultrathin sections were double-stained with lead citrate and uranyl acetate and examined under an electron microscope (100-CX, JEOL, Japan).

Morphometric analysis of glomeruli

Light microscopic images of round or oval-shaped glomeruli (more than 20 glomeruli, original magnification: ×400) stained by PAM staining containing both the poles of blood vessels and tubules (Fig. 1), and free of sclerotic lesions, were digitized using a video grabber (3-CCD CS 5520, Tokyo Electric Industry, Tokyo). Digitized images (final magnification: ×400, resolution: 200 pixels/inch) were analyzed using a digital-tablet (Wacom, Tokyo) for determination of the following parameters using NIH image analysis software (ver. 1.61; NIH, Bethesda, Md., USA). In brief, after digitization of a glomerular image, the outline of the glomerulus was manually traced on the digital tablet, and the area was computed by the image analysis software. For measurement of glomerular diameter, we set circular scales over the glomerulus, and obtained the maximum and minimum diameters of the glomerulus. For loop diameter, we selected opened lumens but excluded deformed and/or shrunken lumens. The following parameters were also computed in each case:

1. Glomerular diameter: the average of the major and minor axes.
2. Glomerular area (AG): the area of the traced glomerulus image.

We also determined the diameter and number of capillary loops. For measurement of the diameter of capillary loops, we selected opened capillaries but excluded capillaries with diagonal lumens and those with deformed lumens.

3. Number of capillaries (Ncap): counting of capillary lumens excluding artificially deformed lumens within the glomerulus.

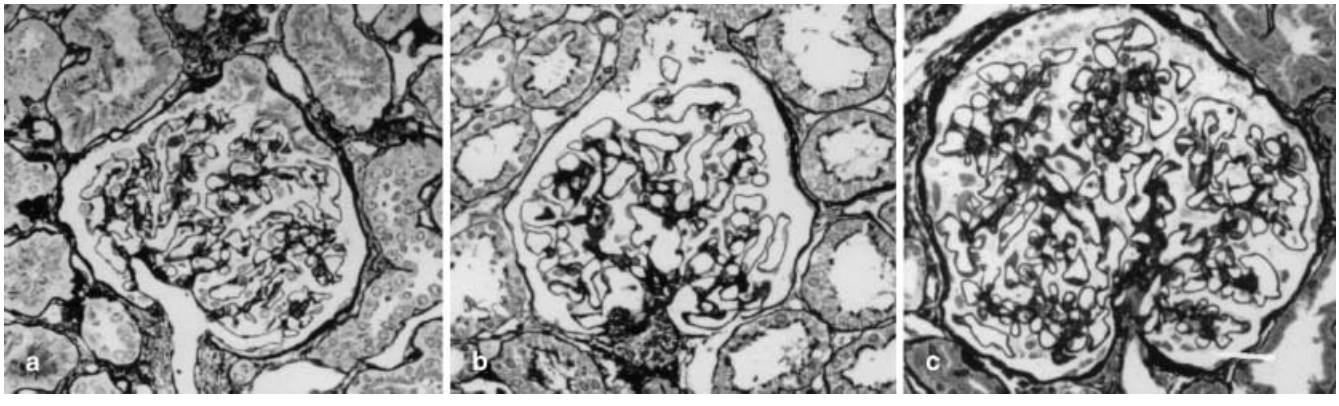

Table 1 Clinical data of VUR patients with good and poor prognosis. Data were obtained at the time of surgery and end of the follow-up period

	Good prognosis		Poor prognosis	
	Baseline	Last	Baseline	Last
Urinary protein (mg/day)	34.8±34.0	27.8±39.2**	514±572*	993±1084*,**
Creatinine-clearance (l/day)	131.0±48.6	117.1±30.2	65.7±27.8**	46.7±24.7**
Serum creatinine (mg/dl)	0.9±0.3**	1.8±2.1	0.4±0.1**	0.6±0.1

Values are mean±SD
*P<0.05, **P<0.01

4. Diameter of open capillaries: the average of the minor axis of selected capillary loops with opened lumen (65–140 capillaries/glomerulus).

We also calculated the mean glomerular tuft volume and glomerular capillary length by using the methods of Weibel [14]. The glomerular tuft volume (V_G) was calculated according to the formula.

5. Glomerular volume: $V_G=(\beta/k)(A_G)^{2/3}$, where $\beta=1.38$ is the shape coefficient and $k=1.1$ is the size distribution coefficient spheres.

Capillary length density (Lvcap) and absolute length of capillaries per glomerulus of average volume (Lcap) were estimated by using previously described formulae [14].

6. Glomerular capillary length (Lcap): $Lvcap=2\times Ncap/(A_T)$ and $Lcap=Lvcap\times(V_G)$, where Ncap, the number of capillary profiles, and A_T, the corresponding tuft area, were determined by (2) and (3) above.

In each case, intact glomeruli (average 20 glomeruli/case) were used for measurement of glomerular diameter and area. Among these, five glomeruli were selected for the measurement of capillary number and diameter. In such glomeruli, the number of podocytes (epithelial cells) was also determined by light microscopy by counting the number of nuclei of podocytes but excluding nuclei of inflammatory cells, mesangial cells and endothelial cells in the field (×400). The number of podocytes (nuclei) per number of capillaries was calculated by use of item (3) above. All analyses were performed by three pathologists who were blinded to the clinical data.

Statistical analysis

All data were expressed as mean±SD. Differences between groups were examined for statistical significance using unpaired and paired Student's *t*-test, ANOVA, Chi-square test, and linear regression analysis. Probability less than 0.05 was considered significant.

Results

Clinical manifestations

Proteinuria was noted in all VUR patients at the time of surgery (baseline); however, it was significantly more severe ($P<0.01$) in patients with poor prognosis than in those with good prognosis (Table 1). At last follow-up, the level of proteinuria in patients with good prognosis showed some improvement, albeit insignificantly, compared to baseline. On the other hand, proteinuria increased by almost two-fold at follow-up in patients with poor prognosis ($P<0.05$). Creatinine clearance at baseline in patients with poor prognosis was lower than in those with good prognosis, albeit insignificantly (Table 1). Furthermore, it decreased in patients with poor prognosis after follow-up period, but not in patients with good prognosis. No difference in serum creatinine level was found during the observation period or between the two prognostic groups (Table 1).

Morphometric changes in glomeruli

During maturation process from 10 to 20 years of age in the control group, glomerular size, including diameter, area and volume, increased exponentially, and reached a plateau during 17–20 years (Fig. 2b), although capillary diameter did not show significant changes (Fig. 2a). Table 2 summarizes the mean values of several glomeruli-related parameters in control subjects and VUR patients with good and poor prognosis. Glomerular volume was highest in patients with poor prognosis (significantly higher than in VUR patients with good prognosis and control cases). The volume tended to be higher in patients with poor prognosis than in control cases, albeit insignificantly (Table 2). A similar trend was also found in the number of glomerular capillaries (Table 2). Capillary diameter was not different among the three groups

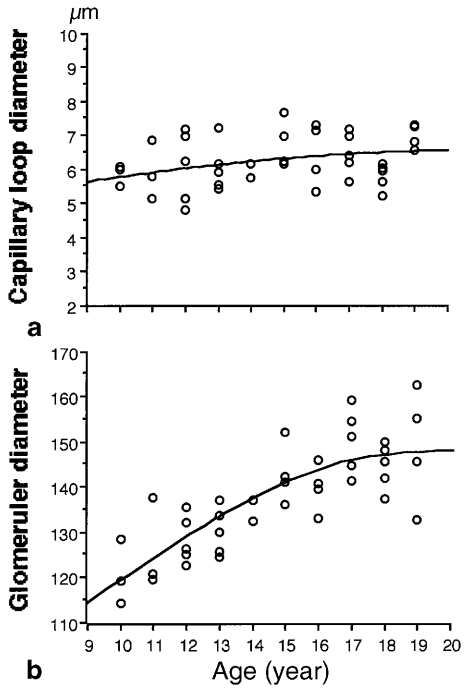

Fig. 2a,b Relationship between age and diameter of capillary and glomerulus in control patients. **a** Relationship between age and capillary diameter. **b** Relationship between age and glomerular diameter

Table 2 Comparison of various morphometric indexes between control cases and VUR patients with good and poor prognosis

	Control	VUR	
		Good prognosis	Poor prognosis
Glomerulus			
Diameter	115.6±15.1	120.5±16.7	156±20.4*,**
Area	1.20±0.2	1.57±0.5	3.17±1.1*,**
Volume	1.98±0.4	2.37±0.5	5.54±0.7*,**
Glomerular capillary			
Number	65.1±10.0	75.3±20.1	138.2±49.2*,**
Diameter	6.1±0.6	5.0±0.5	5.3±0.4
Length	18.8±5.0	24.3±7.5	61.6±23.5*,**

Values are mean±SD
* $P<0.01$ in VUR patients with good prognosis
** $P<0.01$ greater compared to control cases

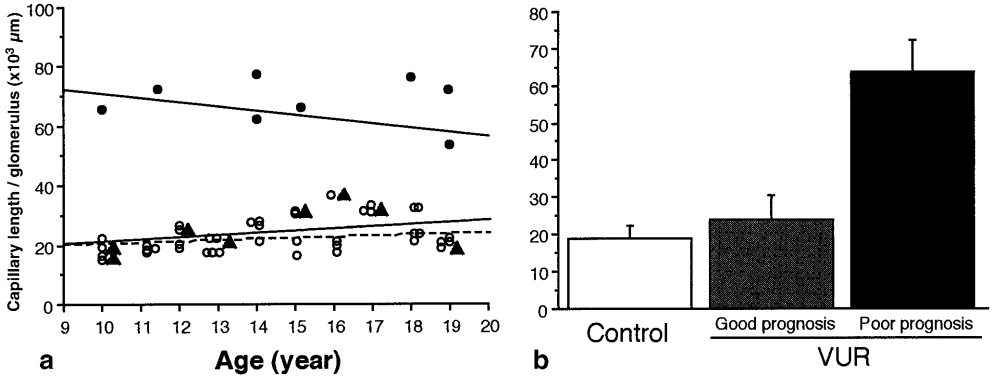

Fig. 3a,b Capillary length and relationship with age. **a** Relationship between age and glomerular capillary length/glomerulus in control cases with normal renal function (*open circles*) and VUR patients with good prognosis (*closed triangles*) and poor prognosis (*closed circles*). **b** Glomerular capillary length in control cases and VUR patients with good or poor prognosis. Values are mean±SD; **P*<0.01 versus the control

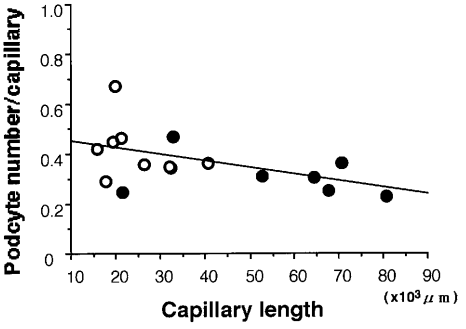

Fig. 4 Relationship between capillary length and podocyte number/capillary in VUR patients. There was a significant negative correlation between capillary length and the number of podocytes/capillary in patients with VUR with good prognosis (*open circles*) and poor prognosis (*closed circles*) (*P*<0.01)

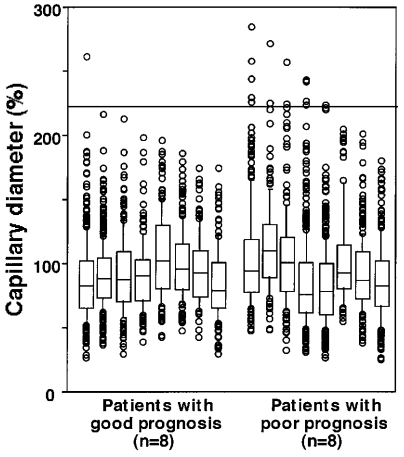

Fig. 5 Appearance of focally expanded capillary segments in VUR patients with good and poor prognosis. Capillary diameter in each VUR patients with good and poor prognosis, as shown by percent of mean capillary diameter of control cases. The appearance of expanded capillary segment is noted in patients with poor prognosis compared to patients with good prognosis

(Table 2). However, the total capillary length was longest in patients with poor prognosis, followed by those with good prognosis (Fig. 3b). We also examined the relationship between capillary length and age. The slope of such relationship was positive in the control (r=0.30) and patients with poor prognosis (r=0.39, Fig. 3a), but negative in patients with poor prognosis (r=0.593).

The number of podocytes/capillary correlated negatively and significantly with glomerular capillary length in VUR patients (P<0.01, Fig. 4). The number of podocytes/capillary in patients with poor prognosis (0.44 podocytes/capillary) was significantly (P<0.01) lower than in patients with good prognosis (0.53 podocytes/capillary). Furthermore, focally (cystically) expanded capillaries with a mean diameter exceeding 13.7 μm [≥225% of the mean diameter of age-matched control (6.1 μm)] were detected in five of eight patients with poor prognosis compared to only one patient among those with good prognosis (Fig. 5). Cystically expanded capillaries were relatively few in number and did not influence the mean capillary diameter in each group.

Ultrastructural changes in glomeruli

We also examined electron microscopically at least three intact glomeruli in each of the control and VUR cases, and applied morphometric analysis to examine differences between the groups. Although mesangioproliferative and sclerotic lesions in glomeruli were not detected in any case, two pathological changes were found in five VUR cases with poor prognosis (Table 3). These consisted of podocyte detachment (Fig. 6a) and tuft adhesion to the Bowman's capsule (Fig. 6b). These changes were not seen in control patients and in only one patient with good prognosis (Table 3).

Table 3 Podocyte detachment and adhesion of capillary tufts to Bowman's capsule in VUR patients with good and poor prognosis

	Control	VUR	
		Good prognosis	Poor prognosis
Glomerular injury			
Podocyte detachment	0	1	4
Adhesion to Bowman's capsule	0	0	2
Positive cases/total case (%)	0/40 (0)	1/8 (12.5)	5/8 (62.5)*

Data represent number of cases in each group
**P*<0.05 versus control and VUR patients with good prognosis

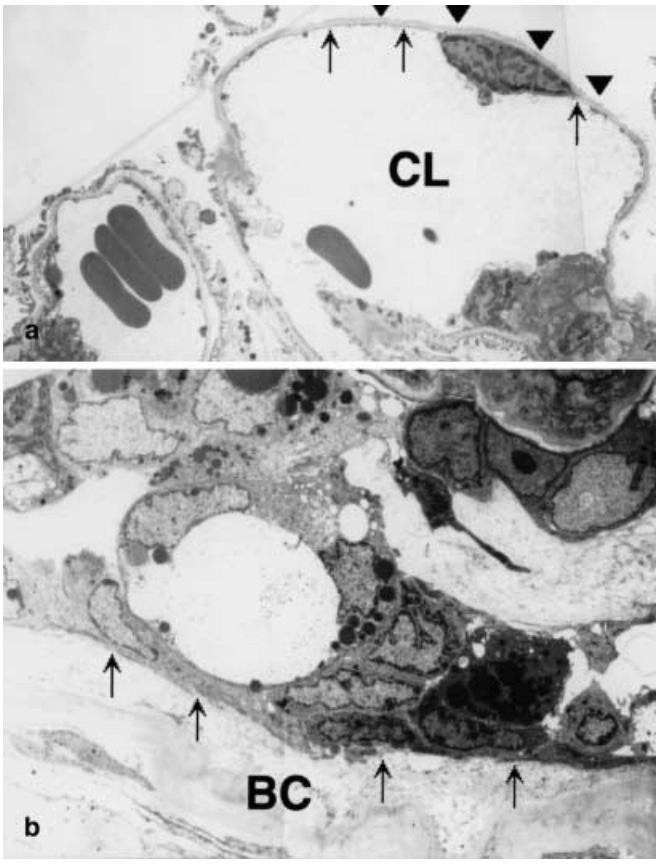

Fig. 6a,b Electron micrographs of glomeruli from VUR patients with poor prognosis. **a** Podocytes are denuded (*arrowheads*) from the basement membrane of glomerular capillary loop (*arrows*) in a patient with VUR and poor prognosis (15-year-old male). *CL* capillary lumen. ×2000. **b** The glomerular capillary adheres (*arrows*) to Bowman's capsule (*BC*) in a patient with VUR and poor prognosis (13-year-old male). ×4600

Discussion

We have demonstrated in the present study several histopathological changes in young patients with VUR. These included extensive elongation of glomerular capillaries accompanied by a corresponding increase in glomerular volume. Such changes were associated with poor prognosis within 10 years after surgery for VUR. VUR is a suitable human model for investigating glomerular changes induced by mechanical stresses, such as hyperfiltration. Our findings are consistent with previous studies demonstrating increased glomerular volume in human cases with RN [4, 5, 6, 7,8]. It has been postulated that remnant glomeruli can compensate for the collapsed dysfunctional nephron, resulting in glomerular hypertrophy and focal segmental glomerular sclerosis (FSGS) [4, 6, 8] or global sclerosis [2]. However, the exact pathological changes occurring in the glomeruli before the development of FSGS in human cases with VUR have not been previously investigated except for glomerular hypertrophy.

In our selected patients, renal biopsies were obtained from non-scarring regions of the kidney. FSGS lesions were not detected microscopically in all cases. Previous studies have shown that in RN [6, 15] as well as in other glomerular diseases [14, 16], the development of glomerular sclerosis accompanied by severe proteinuria is a strong predictor of poor renal prognosis. Thus, our patients were still in an early stage of compensatory glomerular hypertrophy rather than in a state of pending sclerosis. In such patients, we investigated both early pathological changes in glomeruli and the relationship between such changes and prognosis.

Yoshiara et al. [8] reported that glomerular hypertrophy in young patients with RN (age 5.2–18.8 years) was associated with a two-fold increase in the tuft area. In the absence of mesangial proliferation, two processes might contribute to the development of glomerular hypertrophy. These include elongation of capillaries [12] and expansion of the capillary lumen [11, 17]. Using a rat model of compensatory renal hypertrophy, Schwartz et al. [12] demonstrated increased capillary length without an increase in capillary radius. At young age, glomeruli may show a strong adaptation capacity [15, 18]. On the other hand, glomerular hypertrophy is known to aggravate podocyte injury in nephrotic rats [19]. Similar changes were detected in our study, and there were two types of young patients with VUR who showed extensive increase in capillary length (Fig. 3b). The present retrospective study demonstrated that patients who later showed poor renal prognosis exhibited a larger increase in capillary length (3.31-fold), compared to patients with good prognosis (1.25-fold) relative to the respective length of control. In this regard, capillary length is known to increase as a result of hyperfiltration, as demonstrated previously in an animal study following nephrectomy [12]. Under these conditions, renal function, however, remains intact and shows no deterioration [20].

Regression analysis in the present study (Fig. 3) showed that patients with good prognosis tended to maintain a stable capillary length among the different ages examined in our study, similar to control subjects. In contrast, patients with poor prognosis showed an age-related deterioration of total capillary length. Experimental studies have shown that functional glomerular hypertrophy accompanied by capillary lengthening is often reversible following surgery, but the capillary length is not [12]. Thus, it is possible that the extent of capillary lengthening in patients with good prognosis may be within the physiological range of response at young age. On the other hand, additional mechanisms may be involved in the marked increase in glomerular capillary length (more than three times) seen in patients with poor prognosis.

We also examined the effects of increased capillary length on the number of epithelial cells (podocytes). Several investigators have postulated that podocytes are highly differentiated cells and are unable to complete cell division [9, 21, 22, 23]. Furthermore, their number is fixed after 5 weeks of age and remains stable during nor-

mal and hypertrophic glomerular growth [21]. It has been shown that podocytes are responsible for regulation of albuminuria [10], segmental sclerosis [21], and capillary wall distension [11]. In our study, the number of podocytes/capillary correlated negatively with increased capillary length in cases with VUR. This finding suggests the degeneration or loss of podocytes in VUR patients with elongated capillary beds. We also examined the diameter of each capillary in the expanded glomeruli of VUR cases. Our results showed only few expanded capillaries (≤10%), but their number was higher in patients with poor prognosis than in those with good prognosis (Fig. 5). These results suggest that in patients with poor prognosis, the stress level (probably caused by hyperfiltration) might be beyond the threshold of capillary extension, resulting in severe expansion (more than twice the normal diameter) in some capillary segments. Such regions are likely to exhibit podocyte loss. However, the exact mechanisms remain unknown.

We also examined for regions showing glomerular injury in our patients, i.e. detachment of podocytes from the basement membrane and adhesion of capillary tufts to Bowman's capsule. These changes (Fig. 6), which are considered as common initial changes during the development of glomerular sclerosis in other glomerular disease with nephrotic syndrome [19], were frequently detected in VUR patients with poor prognosis but not in those with good prognosis.

In conclusion, we defined in the present study the initial glomerular changes in patients with RN induced by mechanical stress. Our results showed excessive lengthening of glomerular capillaries and expansion in some capillary segments in patients with poor prognosis. These mechanically induced changes in VUR patients with poor prognosis may subsequently induce podocyte detachment and adhesion of capillary tufts to Bowman's capsule. These initial pathologic changes in the glomeruli may be important in the subsequent development of glomerular sclerosis in patients with VUR.

Acknowledgement This work was partially supported by grants-in-aid from the Ministry of Education, Science and Culture of Japan (No. 05671344 for K.O.).

References

1. Bailey RR (1973) The relationship of vesico-ureteric reflux to urinary tract infection and chronic pyelonephritis-reflux nephropathy. Clin Nephrol 1:132–141
2. Dillon MJ, Goonasekera CD (1998) Reflux nephropathy. J Am Soc Nephrol 9:2377–2383
3. Smellie JM, Poulton A, Prescod NP (1994) Retrospective study of children with renal scarring associated with reflux and urinary infection. BMJ 308:1193–1196
4. Matsuoka H, Oshima K, Sakamoto K, Taguchi T, Takebayashi S (1994) Renal pathology in patients with reflux nephropathy. The turning point in irreversible renal disease. Eur Urol 26: 153–159
5. Akaoka K, White RH, Raafat F (1995) Glomerular morphometry in childhood reflux nephropathy, emphasizing the capillary changes. Published erratum in Kidney Int 47:1108–1114
6. Becker GJ, Kincaid Smith P (1993) Reflux nephropathy: the glomerular lesion and progression of renal failure. Pediatr Nephrol 7:365–369
7. Morita M, Yoshiara S, White RH, Raafat F (1990) The glomerular changes in children with reflux nephropathy. J Pathol 162:245–253
8. Yoshiara S, White RH, Raafat F, Smith NC, Shah KJ (1993) Glomerular morphometry in reflux nephropathy: functional and radiological correlations. Pediatr Nephrol 7:15–22
9. Nagata M, Scharer K, Kriz W (1992) Glomerular damage after uninephrectomy in young rats. I. Hypertrophy and distortion of capillary architecture. Kidney Int 42:136–147
10. Laurens W, Battaglia C, Foglieni C, De Vos R, Malanchini B, Van Damme B, Vanrenterghem Y, Remuzzi G, Remuzzi A (1995) Direct podocyte damage in the single nephron leads to albuminuria in vivo. Kidney Int 47:1078–1086
11. Kriz W, Hackenthal E, Nobiling R, Sakai T, Elger M, Hahnel B (1994) A role for podocytes to counteract capillary wall distension. Kidney Int 45:369–376
12. Schwartz MM, Churchill M, Bidani A, Churchill PC (1993) Reversible compensatory hypertrophy in rat kidneys: morphometric characterization. Kidney Int 43:610–614
13. Lebowitz RL, Olbing H, Parkkulainen KV, Smellie JM, Tamminen Mobius TE (1985) International system of radiographic grading of vesicoureteric reflux. International Reflux Study in Children. Pediatr Radiol 15:105–109
14. Weibel ER (1979) Stereological methods, practical methods for biological morphometry. Academic Press, London, pp 1–415
15. el Khatib MT, Becker GJ, Kincaid Smith PS (1987) Morphometric aspects of reflux nephropathy. Kidney Int 32:261–266
16. Muda AO, Feriozzi S, Cinotti GA, Faraggiana T (1994) Glomerular hypertrophy and chronic renal failure in focal segmental glomerulosclerosis. Am J Kidney Dis 23:237–241
17. Kriz W, Elger M, Nagata M, Kretzler M, Uiker S, Koeppen Hageman I, Tenschert S, Lemley KV (1994) The role of podocytes in the development of glomerular sclerosis. Kidney Int Suppl 45:S64–72
18. Bhathena DB, Julian BA, McMorrow RG, Baehler RW (1985) Focal sclerosis of hypertrophied glomeruli in solitary functioning kidneys of humans. Am J Kidney Dis 5:226–232
19. Miller PL, Scholey JW, Rennke HG, Meyer TW (1990) Glomerular hypertrophy aggravates epithelial cell injury in nephrotic rats. J Clin Invest 85:1119–1126
20. Bidani AK, Mitchell KD, Schwartz MM, Navar LG, Lewis EJ (1990) Absence of glomerular injury or nephron loss in a normotensive rat remnant kidney model. Kidney Int 38:28–38
21. Nagata M, Kriz W (1992) Glomerular damage after uninephrectomy in young rats. II. Mechanical stress on podocytes as a pathway to sclerosis. Kidney Int 42:148–160
22. Rennke HG (1994) How does glomerular epithelial cell injury contribute to progressive glomerular damage? Kidney Int Suppl 45:S58–63
23. Nagata M, Yamaguchi Y, Komatsu Y, Ito K (1995) Mitosis and the presence of binucleate cells among glomerular podocytes in diseased human kidneys. Nephron 70:68–71