

## Light and Electron Microscopic Observation in a Case of Congenital Renal Proximal Tubular Dysfunction

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### ABSTRACT

We report a case of congenital renal proximal tubular dysfunction (CRPTD) accompanied by IgA nephropathy. The mesangial matrix was slightly increased with depositions of IgA and C3. Podocytes contained many clear vacuoles. Cuboidal cells, as well as squamous cells, lined the parietal epithelium of Bowman's capsule, although the functional or pathological significance of the cuboidal cells is unknown. The proximal tubular cells appeared to have a less-developed apical endocytic complex, basal infoldings and fewer lysosomes. These morphological changes may reflect proximal tubular dysfunction.

**Key words:** Congenital renal proximal tubular dysfunction, IgA nephropathy, Bowman's parietal epithelium, Renal proximal tubule

A new pediatric disease entity called 'congenital renal proximal tubular dysfunction' (CRPTD) has recently attracted attention<sup>14,20,24,27</sup>. In this disease, large amounts of low molecular weight proteins (LMWP) are excreted in the urine<sup>18</sup> by malfunction of the reabsorption mechanism in renal proximal tubules. Although it is now widely accepted that CRPTD has no specific pathological changes<sup>14,27</sup> without deteriorating glomerular function, the case reported here shows some morphological changes which have not been reported in light and electron microscopy. We describe these changes with reference to the literature.

### CASE REPORT

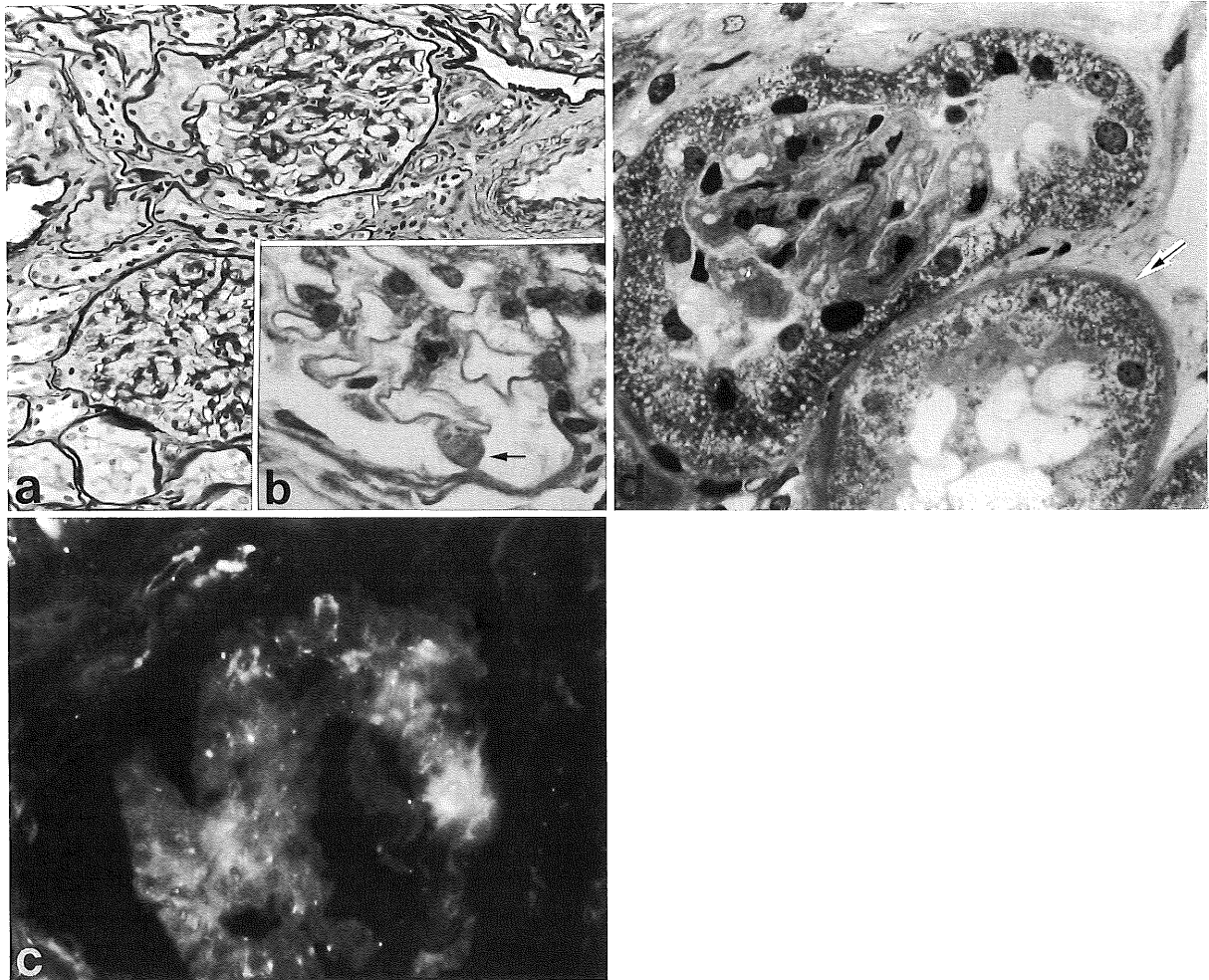
A 20-year-old male was admitted to Hiroshima University Hospital for evaluation of proteinuria was first detected at the age of 18 months. The moderate proteinuria was not responsive to steroids. Each school year, proteinuria was detected yearly, although he was not treated. More precise data are unknown. On February 19th 1987, at the age of 19 years, he was admitted and underwent a renal biopsy. The biopsy specimen revealed mild mesangial cell proliferation by light microscopy and with IgA and C3 deposition in the mesangial area demonstrated by immunofluorescence study. He was diagnosed as having mild IgA nephropathy with normal glomerular function and

was treated with dipyridamole (300mg/day, p.o.). On August 4, 1988 at the age of 20 years, he was readmitted for further evaluation because of increasing urinary  $\beta$ -2-microglobulin ( $\beta$ 2-m) levels (8411ng/ml).

On admission, the physical examination was unremarkable with a blood pressure of 112/48mmHg, body weight of 59kg, and a height of 170cm. Mental development was normal. Urinalysis revealed proteinuria of 100mg/dl, trace occult blood and 5-10 white and red blood cells per high-power field with a few hyaline granular casts. The 24-hour urinary protein was 1-2g. Peripheral blood examination revealed a normal RBC ( $471 \times 10^4/\text{mm}^3$ ), hemoglobin (15.1g/dl), WBC ( $4700/\text{mm}^3$ ) and differential, platelet count ( $23.4 \times 10^4/\text{mm}^3$ ) and sedimentation rate (7mm/h). Blood coagulation studies and liver function tests were all normal. The serum protein was 7.7g/dl, the serum albumin was 4.5g/dl and the total cholesterol 174mg/dl. Serological tests revealed an elevated IgA (363mg/dl) and normal levels of IgG (1500mg/dl), IgM (117mg/dl), C3 (47mg/dl) and C4 (22mg/dl). Renal function tests showed normal serum creatinine (0.88mg/dl), blood urea nitrogen (15mg/dl), uric acid (3.2mg/dl), creatinine clearance (117ml/min), and GFR (84ml/min), but slightly decreased levels of RPF (349ml/min) and RBF (618ml/min). The results of both PSP (15 min) and Fishberg test

**Table 1.** Serum and urinary low molecular weight proteins

	serum	urine
$\beta$ 2-microglobulin	1910.4 ng/ml	31773.8 ng/ml
Lysozyme	11.8 $\mu$ g/ml	94.2 $\mu$ g/ml
Retinol-binding-protein	5.0 mg/dl	8.0 mg/dl
$\alpha$ 1-microglobulin	Not done	67.9 $\mu$ g/ml
$\alpha$ 1-acid glycoprotein	Not done	28.0 $\mu$ g/ml



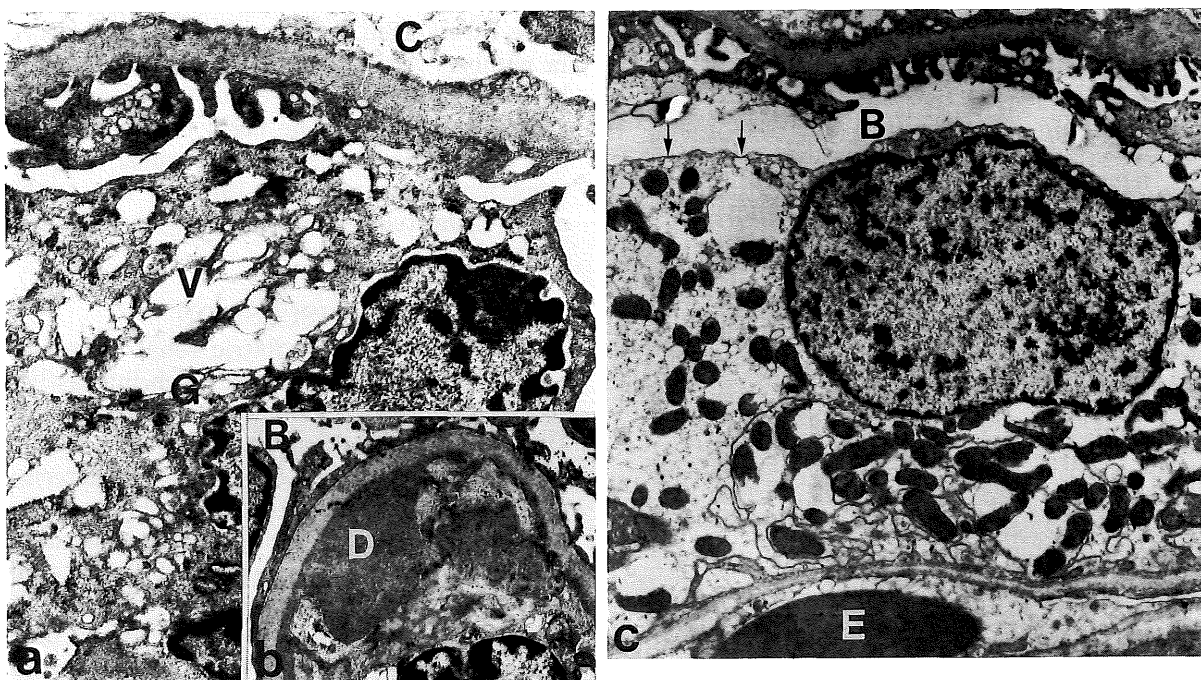
**Fig. 1.** (a) A light micrograph of the renal cortex. PAS-hematoxylin staining.  $\times 190$ . (b) A PAS-positive hemispherical deposit (arrow) is seen in the paramesangial area of a glomerulus. PAS-hematoxylin staining.  $\times 760$ . (c) Immunofluorescence microscopy using anti-IgA serum. (d) A photomicrograph of the semi-thin sections of Epon-embedded specimens. Arrow=Tubular basement membrane. Toluidine blue staining.  $\times 720$ .

were normal with values of 30.7% and 1.025, respectively, but NAG, the enzyme derived from renal tubules, was slightly elevated (15.9U/l) and urinary  $\beta$ 2-m clearance was significantly increased (16.7ml/min). The threshold of maximum glucose reabsorption was decreased (108.8mg/min). Serum and urinary electrolytes were within normal limits. As urinary  $\beta$ 2-m was abnormally high (8411ng/ml) during hospitalization, the urinary LMWP were determined (Table 1) revealing elevated  $\beta$ 2-m, lysozyme, retinol-binding-protein,  $\alpha$ 1-microglobulin and  $\alpha$ 1-acid glycoprotein. The fraction pattern of urinary proteins using cellulose-acetate membrane

electrophoresis showed an elevated fraction (28.1%). A study of urinary  $\beta$ 2-m excretion in the patient's family revealed an elevated value (2045ng/ml) in his mother. IVP and abdominal echogram revealed no morphological abnormalities of the kidneys.

#### PATHOLOGICAL FINDINGS

Tissue block specimens were obtained by percutaneous renal biopsy. For light microscopic study, serial sections were cut from each block and every 6 sections were examined. Staining procedures included hematoxylin and eosin (H&E), periodic acid-Schiff (PAS) reaction, periodic acid-silver methena-



**Fig. 2.** (a) An electron micrograph of a podocyte. V=Vacuoles. G=Golgi complex. C=Lumen of the glomerular capillary.  $\times 13000$  (b) An electron-dense amorphous deposit (D) is seen in the mesangial area. B=Bowman's space.  $\times 6900$ . (c) An electron micrograph of a cuboidal cell in the parietal epithelium of Bowman's capsule. Arrows=Small clear vesicles. B=Bowman's space. E=An erythrocyte in the capillary.  $\times 7300$ .

mine (PAM), and Massons' trichrome staining. Immunofluorescence studies were performed on cryostat sections of snap-frozen materials using antisera to IgG, IgA, IgM, C3, C4, and fibrinogen (Behring, Germany), respectively. For electron microscopy, some specimens were fixed in 2.5% glutaraldehyde, and postfixed in 2% osmium tetroxide, dehydrated and embedded in Epon 812. Semi-thin sections were stained with toluidine blue and thin sections were doubly stained with uranyl acetate and lead citrate.

Light microscopic examination of kidney biopsy specimens revealed mild diffuse proliferation of mesangial cells and matrix in all glomeruli (Fig. 1a). A few hemispherical deposits, stained in pink by the PAS reaction, were seen in the paramesangial area (Fig. 1b). PAM-stained sections showed neither thickening nor duplication of the basement membrane. Granular fluorescence patterns were demonstrated in the mesangial area by immunofluorescence microscopy, using anti-IgA (Fig. 1c) and anti-C3 serum, but negative staining was obtained using anti-IgG, anti-IgM, anti-C4 or anti-fibrinogen serum. Among a total of 47 renal corpuscles observed, eight had cuboidal cells along with the usual squamous cells lining the parietal layer of Bowman's capsule. In some renal corpuscles, the cuboidal cells were located near the urinary pole so that the capillary loops of the glomerulus appeared to protrude into the orifice of the proximal tubule. However, the cuboidal cells were distinguished from proximal tubular cells by

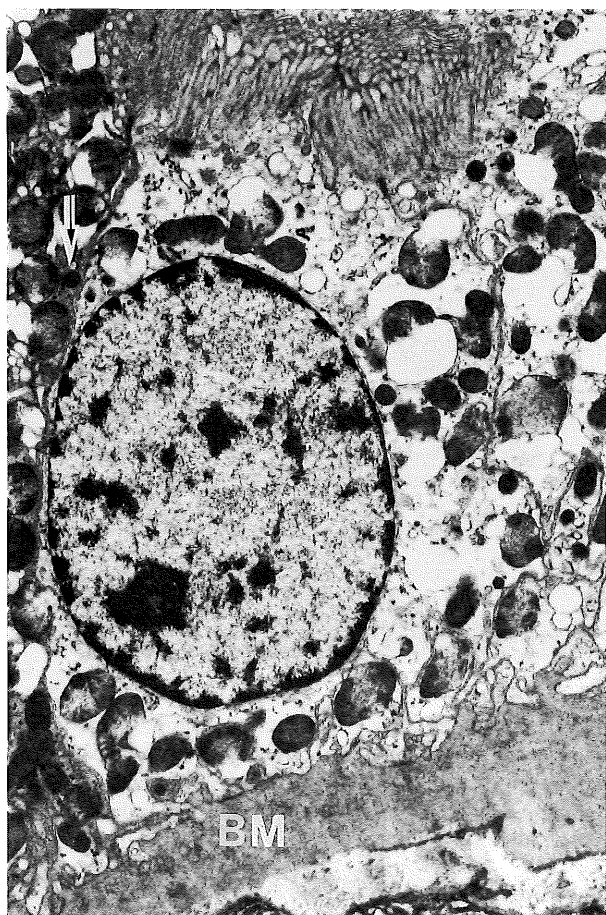
their lack of a PAS-positive brush border. In some Bowman's capsules, the cuboidal cells were seen apart from the urinary pole and were intermingled with squamous epithelial cells.

The cuboidal cells were more apparent under light microscopic examination of the semi-thin sections of Epon-embedded specimens (Fig. 1d). As observed in the paraffin-embedded sections, cuboidal cells could be seen lining Bowman's capsule in 3 of 6 renal corpuscles. The cuboidal cells were located both in the vicinity of the urinary pole and other parts of Bowman's capsule.

Urinary tubules appeared almost normal, except that the PAS-positive basement membrane seemed thicker around the proximal convoluted tubules.

In electron microscopic observation, occasional thinning of the basement membrane was observed in the glomerulus. Podocytes contained many clear vacuoles of 50-920nm in diameter (Fig. 2a). These vacuoles seemed to be closely related to the Golgi complex. A flattening of foot processes was sometimes seen. In the mesangial area, a small number of electron-dense amorphous deposits were seen (Fig. 2b).

Cuboidal cells were also detected by electron microscopy in the parietal epithelium of Bowman's capsule (Fig. 2c). The round nucleus of the cuboidal cell was located near the cell apex. The apical cell membrane rarely projected microvilli, but was sometimes slightly scalloped. The apical cytoplasm contained small clear vesicles. Numerous oval mitochondria were conspicuous in the cytoplasm. At



**Fig. 3.** An electron micrograph of proximal tubular cells. Arrows=Lysosomes. BM=Basement membrane.  $\times 7400$ .

the cell base, the basal cell membrane infolded among mitochondria, although the basal infoldings were not as developed as those of the proximal and distal convoluted tubular cells.

The usual squamous epithelial cells of Bowman's parietal layer had an almost normal appearance. They contained sparse cytoplasmic organelles such as the endoplasmic reticulum, small mitochondria, small Golgi complex and small clear vesicles. Clear vacuoles, as seen in podocytes, were rarely found. Cells showing intermediate morphology between normal squamous epithelial cells and the cuboidal cells were not found in this study.

The basement membrane of the parietal layer of Bowman's capsule was highly variable in thickness (240–970nm). The basement membrane attached to the cuboidal cells appeared thinner (240–370nm) than that of other parts (630–940nm).

The proximal tubular cells had a normal cuboidal shape (Fig. 3). The apical cell surface projected numerous microvilli to form a brush border. Normal proximal tubular cells usually contain the apical endocytic complex, consisting of many tubules, vesicles and some endosomes. However, in this patient, elements of the apical endocytic complex appeared decreased. The apical cytoplasm con-

tained some vesicles, but tubules and endosomes were rarely seen. Lysosomes were also decreased in number. Although mitochondria were artificially swollen, they were seen in the cytoplasm as in the normal kidney. The basal infoldings appeared shorter. The thickness of the proximal tubular basement membrane was 680–1980nm. The epithelium of the distal tubules and collecting ducts showed no pathological changes.

## DISCUSSION

CRPTD is a new pediatric renal disease entity which has recently attracted much interest<sup>14,20,24,27</sup>. It was first reported in Japan by Okada et al<sup>24</sup> in 1984. Kobayashi et al<sup>14</sup> and later Geary et al<sup>10</sup> reported similar cases. CRPTD is characterized by massive urinary excretion of LMWP (molecular weight less than 40,000). In the normal state, LMWP passes almost freely through the glomerular capillary wall, and more than 99% of filtered LMWP is reabsorbed and catabolized by proximal tubular cells<sup>12,16,18,27</sup>. Thus, urinary excretion of LMWP suggests a certain failure in reabsorption. CRPTD differs from well-known renal tubular diseases such as Fanconi's syndrome<sup>17</sup>, renal tubular acidosis<sup>8</sup>, and heavy metal poisoning<sup>13</sup>, because it has neither other metabolic disorders nor any mental or physical developmental disturbances. In addition, the histology of the renal tubules has been reported normal in the majority of CRPTD cases<sup>14,27</sup>.

In the nation-wide survey in Japan conducted by Murakami et al<sup>22</sup> in 1987, 53 cases of CRPTD were confirmed. Criteria for diagnosis of CRPTD were proposed by Murakami et al (Table 2). In the present case, SDS-PAGE method was not performed; instead the LMWP such as  $\beta 2$ -m, lysozyme, retinol-binding-protein,  $\alpha 1$ -microglobulin and  $\alpha 1$ -acid glycoprotein were measured and found significantly higher. The present case satisfied the criteria for the diagnosis of CRPTD.

Murakami et al examined 32 cases of CRPTD histopathologically. IgA deposition in the mesangial area was observed by immunofluorescence in only one of 26 cases. Except for a relatively high incidence of FGS and PAS-positive intratubular casts, no specific pathological changes were present in the glomeruli or tubules of CRPTD patients<sup>22</sup>. Other authors have also reported a lack of specific pathological changes in CRPTD<sup>14,27</sup>.

In the present case, a slight proliferation of mesangial cells in the glomerulus and IgA deposition in the mesangial area was observed. However, it is not unlikely that these findings are less attributable to CRPTD than to an accompaniment of IgA nephropathy<sup>11</sup>. When the IgA nephropathy first occurred is not clear, but that IgA nephropathy occurred after CRPTD as presentation of IgA nephropathy at 18 months of age is unlikely<sup>15</sup>.

In addition to changes in the glomeruli as

**Table 2.** Congenital renal proximal tubular dysfunction (by Murakami)

1. mild proteinuria
2. occasional hematuria
3. free from mental and physical developmental disturbances
4. male dominant
5. normal renal function during childhood
6. no change in roentgenogram and echogram of the kidney
7. proteinuria with a low percentage of albumin
1) increased urinary excretion of $\beta_2$ -microglobulin
2) urinary excretion of lysozyme
3) increase of urinary $\alpha$ -globulin fraction by cellulose-acetate membrane electrophoresis
4) increase of low molecular weight protein fraction (molecular weight $\leq 40,000$ ) in urine by SDS-PAGE method

described above, we found many clear vacuoles in the podocyte cytoplasm. Some authors reported similar vacuoles in IgA nephropathy<sup>26</sup>. Bergstrand et al<sup>2</sup> found many clear vacuoles in healthy young men and considered the vacuoles related to an increased fluid uptake of the cells; as such, they may not be a sign of renal disease. At present, it is difficult to interpret the meaning of the clear vacuoles in the podocytes.

We observed cuboidal cells lining the parietal layer of Bowman's capsule. Many investigators have reported similar cells in humans and some animals<sup>1,16,19</sup>. In man, similar cases have been reported. Hyperplasia of the proximal epithelial cells is followed by migration into the glomerulus where they characteristically line Bowman's capsule and become cuboidal in renal disease<sup>3</sup>, and other normal<sup>30</sup> and abnormal states<sup>4,9,17,23</sup>. However, almost all of these cells have cytological characteristics of proximal tubular cells, and differ from the cuboidal cells seen in this case.

Cuboidal cells were present both at the urinary pole and apart from it. They lacked a brush border and contained numerous mitochondria. These characteristics are quite different from the usual squamous cells of Bowman's parietal epithelium and proximal tubular cells, and there are no intermediate forms among these three types of cells. At the urinary pole, the demarcation between cuboidal and proximal tubular cells is very sharp, similar to the demarcation between squamous cells of Bowman's parietal epithelium and proximal tubular cells in the normal kidney<sup>28</sup>. Whether these changes are congenital or acquired with the disease is unclear.

In 1981, Andrews<sup>1</sup> reported the presence of proximal tubule-like cells in the parietal epithelium of unilaterally nephrectomized rats. The presence of these cells may be an attempt by the nephron to increase its functional capacity in response to an increased work load. Cells with differing morphological characteristics have been reported in the epithelium of Bowman's capsule in patients with malignancy of other organs. This suggests that atypical epithelial cells in Bowman's capsule are acquired. The cuboidal cells in this patient may represent an attempt to increase reabsorption in response to a massive excretion of LMWP result-

ing from malfunctioning proximal tubular cells, although the cuboidal cells were clearly distinguished from proximal tubular cells. It is still unknown whether these morphological characteristics have any relationship with the massive urinary excretion of LMWP.

It is generally accepted that LMWP which pass through the glomerular capillary wall are absorbed by tubules and endocytic vesicles at the apical border of the proximal tubular cell<sup>16</sup>. The endocytotic vesicles migrate from the apical border to the cell interior and eventually fuse with lysosomes. In this patient, proximal tubular cells seemed to have shorter microvilli, fewer endocytotic vesicles, fewer lysosomes, and shorter basal infoldings than proximal tubular cells in normal kidneys<sup>29</sup>. These changes suggest decreased absorption of LMWP in this patient, and this is a reasonable mechanism for the low molecular weight proteinuria in CRPTD. However, some pathologists believe that detailed study of the tubules in a renal biopsy is a non-productive exercise with little diagnostic value<sup>25</sup>. This is true to an extent, as ultrastructural changes may occur in improperly fixed and processed renal biopsy specimens. Our conclusions are subject to the same concerns, however apical endocytic complex and lysosomes were decreased in this patient. Tubules may appear to be vesicles as an artifact, but the development of the endocytic complex as a whole and the number of lysosomes should not change. Other CRPTD cases should be examined precisely.

CRPTD appears to be a disease with a good prognosis, without deteriorating glomerular function<sup>14</sup>. As a newly defined disease, however, long-term follow-up has not yet occurred and its true prognosis remains unproven. As recently reported by Murakami et al<sup>21</sup>, CRPTD is frequently accompanied by FGS. If, as reported by Churg et al<sup>5</sup>, FGS is a disease of poor prognosis during childhood, CRPTD may show long-term progressive deterioration of glomerular function. The pathophysiology of CRPTD deserves further careful attention.

(Received July 7, 1992)

(Accepted November 4, 1992)

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