

Case Report

## Urothelial hyperplasia with calculi (papillomatosis) in the urinary bladder of a male spontaneous diabetic Torii rat

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**Abstract:** A 40-week-old male spontaneous diabetic Torii rat, an animal model of type 2 diabetes mellitus, was found to have marked urinary calculi with hematuria in the urinary bladder on necropsy. Histological findings in the urinary bladder included a papillary growth pattern with a fibrovascular stroma without atypia. Fine granular materials in the bladder lumen were positive for Von Kossa staining but negative for periodic acid-Schiff or Gram staining, indicating no apparent bacterial infection in the urinary bladder. Scanning electron microscopy revealed that the urinary calculi were magnesium ammonium phosphate crystals (struvite). On the basis of the results, the lesion was diagnosed as urothelial hyperplasia with calculi (papillomatosis). Chronic inciting stimuli by struvite crystals were considered the primary cause of the bladder findings. (DOI: 10.1293/tox.2019-0057; J Toxicol Pathol 2020; 33: 21–24)

**Key words:** the spontaneously diabetic Torii rat, urothelial hyperplasia, urinary calculus, papillomatosis

### Introduction

The spontaneously diabetic Torii (SDT) rat is an animal model of non-obese type 2 diabetes mellitus, which has been established from Sprague-Dawley rats<sup>1</sup>. Male SDT rats exhibit apparent glucose intolerance, glycosuria, and polyuria at 20 weeks of age and spontaneous diabetes with a cumulative incidence of 100% by 40 weeks of age<sup>2</sup>. Chronic hyperglycemia in the rats causes diabetic complications such as retinopathy, nephropathy (e.g., tubular glycogen deposition and mesangial proliferation), and neuropathy (e.g., voiding dysfunction and diarrhea due to autonomic neuropathy)<sup>3</sup>. SDT rats are prone to urinary tract infections, most likely due to the excrement changes. In the present study, we report on a spontaneous lesion in the urinary bladder with marked urinary calculi in a male SDT rat.

### Case Report

A male SDT rat (SDT/Jcl; 40 weeks of age) was purchased from CLEA Japan, Inc. (Tokyo, Japan). The animal was housed individually in a wire mesh cage in an animal room under controlled conditions (temperature, 23 ± 3°C; relative humidity, 30–70%; and 12-h light/12-h dark cycle) and fed pellet food sterilized by irradiation (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) with chlorinated water *ad libitum*. This experiment was conducted in accordance with the methods approved by the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd. (Tokyo, Japan).

The animal was found dead the day after arrival to the laboratory. At necropsy, several urinary calculi with a maximum diameter of 7 mm were observed in the urinary bladder (Fig. 1). They were typically white to yellowish white in color and friable with a smooth surface. The mucosa slightly became thickened with blood. Multiple erosions/ulcers in the glandular stomach, abnormal black contents in the gastrointestinal tract, thymic atrophy, and adrenal hypertrophy were observed.

The urinary bladder was fixed in 10% neutral buffered formalin with or without decalcification prior to processing and embedded in paraffin. Decalcified sections (2 µm thick) were stained with hematoxylin and eosin (H&E). Undecalcified sections (2 µm thick) were subjected to Gram, periodic acid-Schiff (PAS), and Von Kossa staining. Immunohistochemistry was performed on the undecalcified sections using antibodies against cytokeratin AE1/AE3 (1:50 dilution; Agilent Technologies, Santa Clara, CA, USA),

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**Fig. 1.** Gross lesion in the urinary bladder with white to yellowish white uroliths of variable sizes. The bladder lumen is also filled with bloody urine.

Iba-1 (1:1,000 dilution; Fujifilm Wako Chemicals USA Inc., Richmond, VA, USA)<sup>4</sup>, and proliferating cell nuclear antigen (PCNA; 1:200 dilution; Agilent Technologies). The urinary calculi and bladder mucosa samples preserved in 10% neutral buffered formalin were subjected to scanning electron microscopy (S-4500, Hitachi High-Technologies Corp., Tokyo, Japan).

On histopathological examination, numerous structures of eosinophilic or basophilic fine granules and amorphous materials were observed in the bladder lumen (Fig. 2A). The urothelium was diffusely thickened and showed papillary proliferation accompanied by fibrous interstitial tissues and dilated blood vessels (Fig. 2B). The urothelium consisted of three to five layers with increased cellular height and round-to-oval nuclei showing no atypia and few mitotic figures (Fig. 2C). The submucosa contained a small number of lymphocytes and plasma cells as well as focal neutrophilic foci. Some desquamated cells were also found in the lumen.

The fine granules were negative for Gram or PAS staining and positive for Von Kossa staining in the undecalcified sections, which indicated that they were not bacteria but urine sediments including divalent metal ions (Fig. 2D). Most of the desquamated cells in the lumen were positive for cytokeratin AE1/AE3, whereas few were positive for Iba-1 (Fig. 2E and F). Therefore, most of the cells were considered exfoliated transitional epithelium rather than macrophages reactive to the calculus components. In addition, few PCNA-positive cells (0 to 5 nuclei per high-power field at 200 $\times$  magnification) were observed in the urothelium.

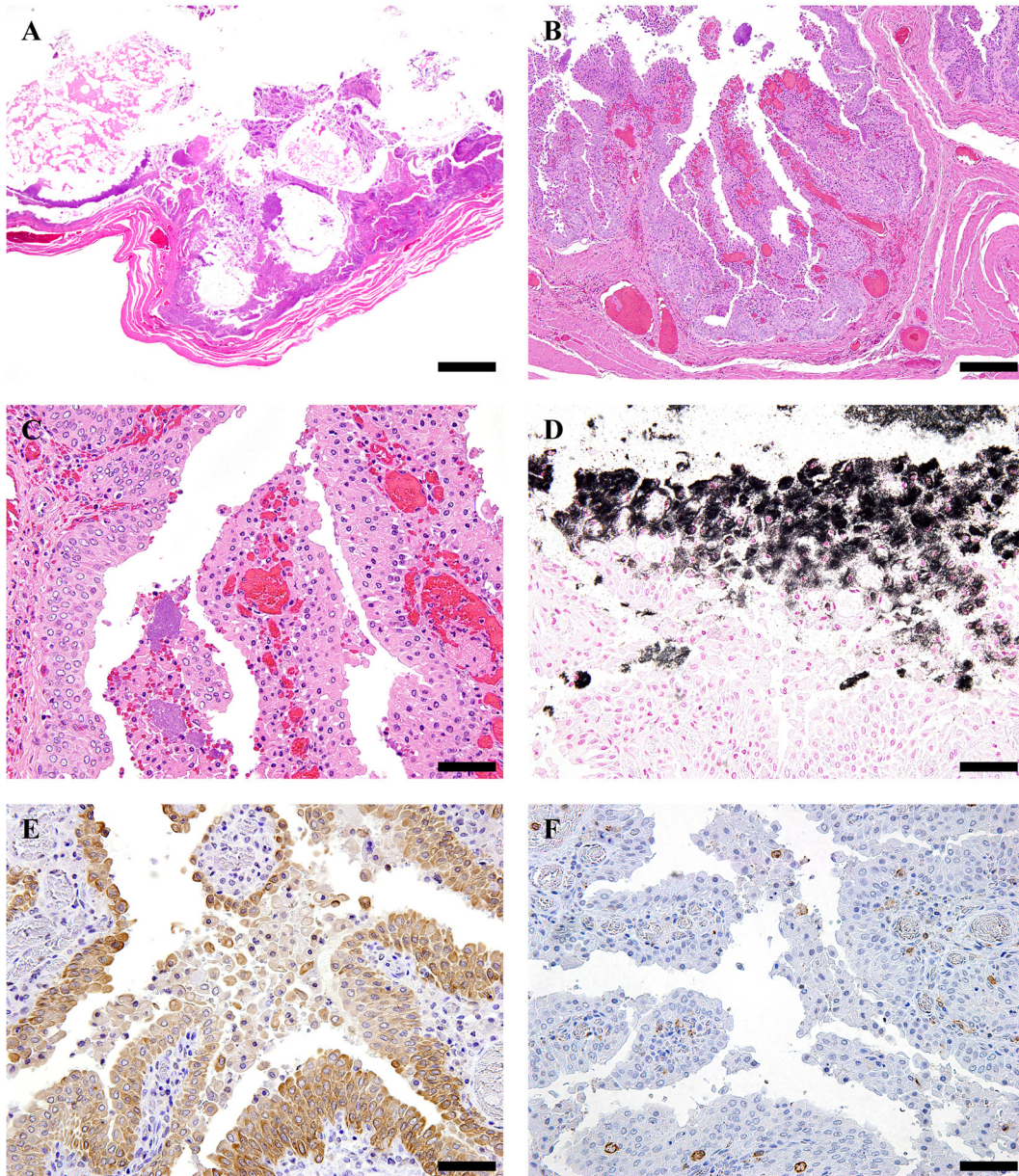
A scanning electron microscopic evaluation of the urinary calculi revealed that these were aggregates of polygonal microcrystals ranging in length from 100 to 200  $\mu\text{m}$  (Fig. 3A). In addition, needle-like microcrystals that were 1 to 5  $\mu\text{m}$  in length were also detected. Based on the ultrastructural characteristics and macroscopic and physical

properties, these urinary solids were considered as magnesium ammonium phosphate crystals (struvite)<sup>5, 6</sup>. This was supported by an elementary analysis using ion chromatography or a flow injection analyzer with urinary calculi (evaluation items: Li, F, Na, Mg, P, S, Cl, K, Ca, Br, and I). Phosphorus and magnesium were detected in the calculi, but calcium was not (data not shown). The luminal surface morphology of the urinary bladder under scanning electron microscope showed slight irregularities or roughness; however, no cluster indicating an ulcer or microorganism was detected (Fig. 3B).

Urothelial hyperplasia is divided into 3 general proliferative patterns: simple, nodular, and papillary<sup>6, 7</sup>. This case was classified as papillary hyperplasia based on the exophytic growth pattern with a fibrovascular core and no epithelial atypia. Shirai *et al.*<sup>8</sup> reported that uracil-induced urolithiasis caused proliferative lesions, including papillary hyperplasia, in rats that were reversible after the withdrawal of uracil and disappearance of the calculi. The lesions are also referred to as “papillomatosis.” In this case, inciting stimuli caused by struvite crystals would have been involved in the urothelial hyperplasia, although no obvious superficial damage or low mitotic activity was observed.

On renal histopathological examination of the SDT rat, glycogen deposition was also found in the tubular epithelium as in previous reports<sup>3, 9</sup>; however, no remarkable change in the glomeruli was observed. In addition, the pelvic epithelium demonstrated pelvic dilation and diffuse hyperplasia along with granular components similar to those in the urinary bladder (data not shown).

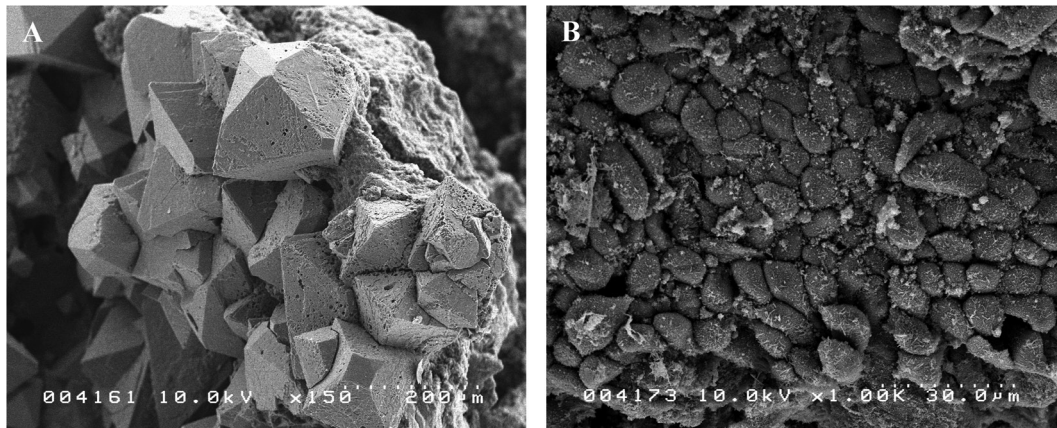
Type 2 diabetes mellitus is considered to increase the risk of nephrolithiasis such as uric acid and calcium oxalate stones due to lower urinary pH and hypocitraturia<sup>10, 11</sup>. In this case, struvite was suggested as the main component of uroliths. Struvite is normally present in rat urine, and infec-



**Fig. 2.** Representative photomicrographs of the urinary bladder. (A) The bladder lumen contained aggregates of granular and amorphous materials. A decalcified and hematoxylin and eosin (H&E)-stained section is shown. Bar = 1 mm. (B) Papillary hyperplasia of the bladder mucosa occurred with stromal fibrous connective tissues and dilated vessels. Decalcified and H&E-stained section. Bar = 200  $\mu$ m. (C) Three to five layers of the transitional epithelium showed increased cellular height without atypia but with few mitotic figures. A few lymphocytes and plasma cells were found in the submucosa. A portion of the mucosal epithelium was exfoliated into the lumen. A decalcified and H&E-stained section is shown. Bar = 50  $\mu$ m. (D) Granular materials in the lumen that were positive for Von Kossa staining. An undecalcified section is shown. Bar = 50  $\mu$ m. (E) Most of the cells in the lumen were positive for cytokeratin AE1/AE3. Bar = 50  $\mu$ m. (F) A few cells in the lumen were positive for Iba-1, which indicated that the cells were generally exfoliated transitional epithelium, not macrophages. Bar = 50  $\mu$ m.

tions with urease-producing bacteria are a significant predisposing factor to increase the risk of struvite formation due to higher urinary pH and urothelial damage by ammonia<sup>6, 12</sup>. The causative factor (e.g., genetic, metabolic, and/or uropathogenic) of the marked struvite stone formation in the SDT rat was unclear, although no apparent bacterial

infection was pathologically indicated in the urinary tract. To the authors' knowledge, this is the first case of papillary hyperplasia with marked struvite uroliths (papillomatosis) in the SDT rat. Background data of urinary calculi and urine assessments, including bacteriological examinations in SDT rats, would reveal the predisposition of the urinary lesion.



**Fig. 3.** Scanning electron microscopy of uroliths and the bladder surface. (A) Urinary calculi composed of aggregates of polygonal microcrystals from 100  $\mu\text{m}$  to 200  $\mu\text{m}$  in length. (B) Surface irregularities and roughness were detected, whereas ulcers and bacteria were not.

**Disclosure of Potential Conflicts of Interest:** The authors declare no conflicts of interest.

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