

## Pathological Characterization of Collagenofibrotic Glomerulonephropathy in a Young Dog

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**ABSTRACT.** This report describes the morphological and immunohistochemical findings of a case of apparent collagenofibrotic glomerulonephropathy in a 7-month-old dog. Clinical examination showed moderate proteinuria with elevated blood urea nitrogen and creatinine. Histopathological examination of the glomerular capillary walls and mesangial areas revealed diffuse and global accumulation of eosinophilic homogeneous or fine fibrous materials, which were immunohistochemically positive for type III collagen. On electron microscopy, the randomly crossed fibrils had transverse bands with a periodicity of approximately 60 nm. The clinical, histopathological, immunohistochemical and electron microscopic findings of the present dog were consistent with those of the human, childhood form of collagenofibrotic glomerulonephropathy.

**KEY WORDS:** canine, collagen, glomerulonephropathy.

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Collagenofibrotic glomerulonephropathy (CFGN) is defined by massive accumulation of atypical type III collagen fibrils within the glomerular extracellular matrix. The pathogenic mechanisms of CFGN remain elusive and this idiopathic glomerular disease is extremely rare in both humans and animals. In humans, the clinical features of CFGN are highly variable and can be divided into two general types based on age at onset of symptoms [6]. The first type typically comprises sporadic symptoms and is detected in adulthood by persistent proteinuria [8]. The severity of clinical symptoms increases slowly and onset of renal dysfunction is delayed. As the majority of reported adult patients have been in Japan, geographic or racial predisposition may be assumed. The other, more severe type begins during childhood and an etiology of autosomal recessive transmission has been suggested [7, 15]. Most patients exhibit progressive proteinuria, eventually leading to nephrotic syndrome.

In animals, glomerulonephropathy similar to CFGN has been reported in dogs [9, 10], a cat [11], pigs [18, 19] and cynomolgus monkeys [1, 5]. There have been two reports of canine CFGN. The first detailed CFGN-like glomerulosclerosis in three Newfoundland dog littermates which were not examined immunohistochemically [10] and the other described CFGN in a 3-year-old Shiba Inu [9]. A genetic disorder is indicated in the former cases, but the cause and pathogenesis of the latter case is unknown.

In this report, we describe the morphological and immunohistochemical characteristics of a juvenile canine case of

glomerulonephropathy with massive deposition of type III collagen fibrils in the glomeruli; a pathology which is consistent with CFGN.

A 7-month-old, male, mix breed (Miniature Dachshund and Chihuahua) dog with growth retardation was referred to a veterinary hospital with depression and hypothermia. Blood analysis revealed high levels of blood urea nitrogen (BUN: >130 mg/dl), creatinine (3.9 mg/dl), phosphate (16.1 mg/dl) and glucose (248 mg/dl), and low levels of calcium (5.5 mg/dl) and albumin (1.8 g/dl) and a low albumin/globulin ratio (0.55). Urinalysis showed moderate proteinuria (reagent strip) and a large number of casts with urogravity of 1.016. The animal died on the day of admission. Necropsy was not permitted, but postmortem nephrectomy was performed for pathological examinations according to the owner's opinion.

The renal specimens were fixed in 10% phosphate-buffered formalin, processed routinely and embedded in paraffin. Histologic sections were cut at 4  $\mu$ m and were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Masson's trichrome (MTC) and Congo red. For immunohistochemistry,  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA; DAKO, Glostrup, Denmark; 1:50) was used as a primary antibody and labeled antigens were visualized with peroxidase-conjugated anti-mouse IgG (Histofine Simple Stain MAX-PO (M); Nichirei, Tokyo, Japan), followed by color development using 3,3'-diaminobenzidine tetrachloride and hydrogen peroxide. Sections were counterstained with Mayer's hematoxylin. For immunofluorescence, the following primary antibodies were used: fibronectin (DAKO, 1:400); fluorescein isothiocyanate (FITC)-conjugated dog IgG (Cappel, Ohio; 1:800); FITC-conjugated dog IgM (BETHYL, Texas; 1:1,000); FITC-conjugated dog IgA

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(BETHYL, 1:500); and dog C3 (BETHYL, 1:1,000). Labeled antigens were directly observed or detected using FITC-conjugated goat anti-rabbit IgG (Cappel) or FITC-conjugated rabbit anti-goat IgG (Cappel) under a fluorescence microscope.

Prior to application of the double-labeled immunofluorescence technique, paraffin sections were dewaxed and pretreated with pepsin solution (Nichirei) for 120 min. Sections were incubated in a mixed solution of mouse anti-type III collagen (Quartett, Berlin, Germany; 1:50) and rabbit anti-type IV collagen (a gift from Dr. K. Arai, Tokyo University of Agriculture and Technology, Tokyo, Japan) overnight at 4°C. After washing with PBS, sections were stained with a mixed solution of FITC-conjugated goat anti-mouse IgG (EY laboratories, Inc., California) and tetramethylrhodamine isothiocyanate (RITC)-conjugated goat anti-rabbit IgG (Gene Tex, Inc., Texas). Normal dog renal

specimens were used as a control.

For electron microscopy, small pieces of formalin fixed renal cortex were refixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were double-stained using uranyl acetate and lead citrate (U/L) or tannic acid and uranyl acetate (T/U), and were then examined using a JEOL 1210 transmission electron microscope (TEM; JOEL, Tokyo, Japan) at 80 kV.

Grossly, glomeruli could be identified on the cut surface of the kidney due to swelling. Microscopically, most glomeruli were enlarged, with edematous or reticular mesangial areas in both kidneys. In addition, diffuse and global accumulation of eosinophilic homogeneous or fine fibrous material was observed in the luminal side of the glomerular capillary walls and mesangial areas (Fig. 1A). This material was stained deep blue with MTC (Fig. 1B), reacted weakly

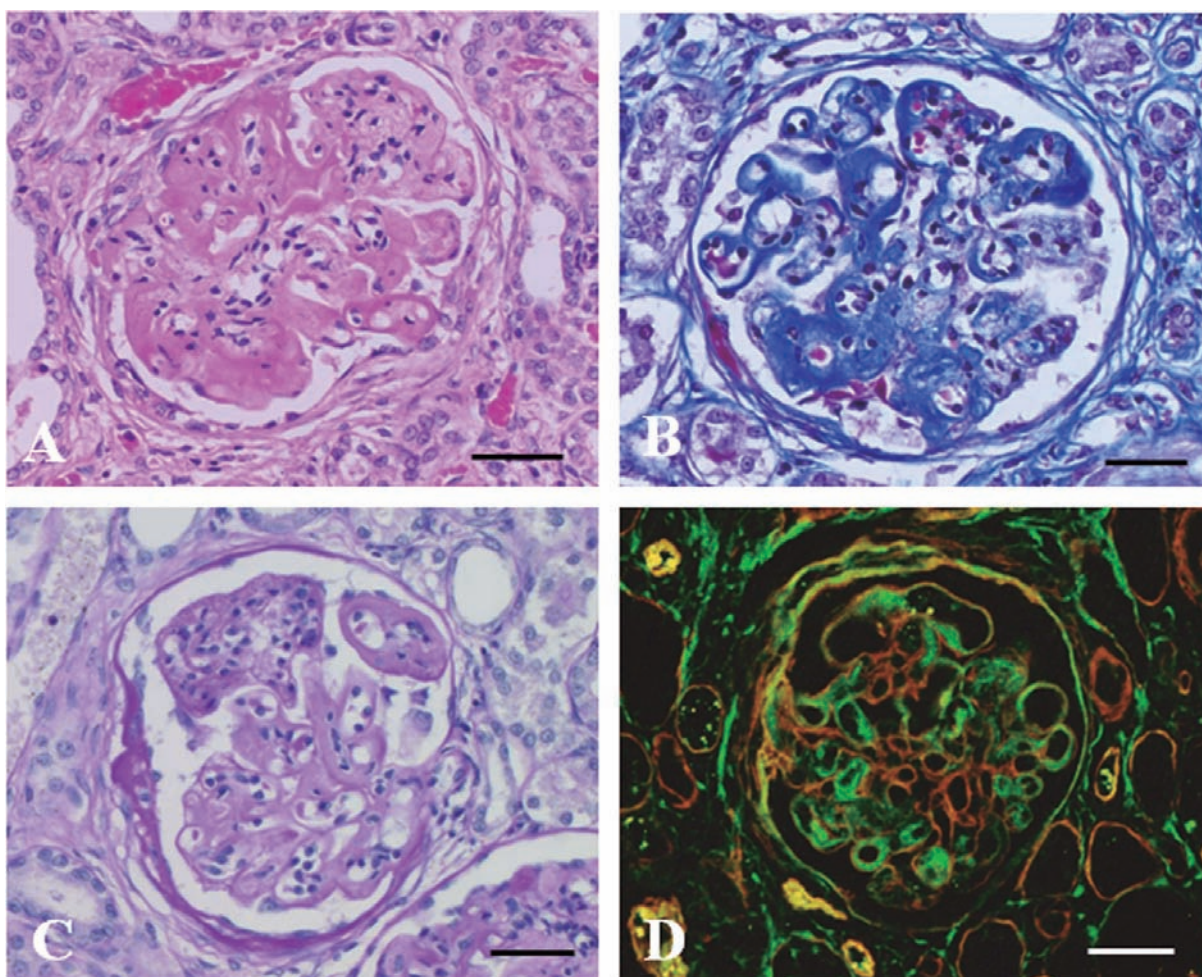


Fig. 1. (A) Global accumulation of eosinophilic homogeneous or fine fibrous materials in the glomerular tufts. Mesangial areas were edematous or reticular appearance. Hematoxylin and eosin (HE) stain. Bar=50  $\mu$ m. (B) Glomerular tufts were diffusely stained deep blue. Masson's trichrome stain. Bar=50  $\mu$ m. (C) Homogeneous deposits in glomerular tufts were weakly positive for PAS and accumulated between the glomerular basement membrane and capillary lumen. PAS. Bar=50  $\mu$ m. (D) Type IV collagen (red) was localized in the glomerular basement membrane and type III collagen (green) was localized in the subendothelial spaces and mesangial areas. Indirect double immunofluorescence. Bar=50  $\mu$ m.

for PAS (Fig. 1C), and was negative for Congo red staining (data not shown). The capillary lumens were frequently compressed by massive accumulation of this material. Sub-endothelial deposits occasionally gave peripheral capillary walls a double-contour appearance. In the glomeruli, rare adhesion of glomerular tufts to the Bowman's capsules, cellular crescent formation and occasional hypercellularity and karyorrhexis were noted. No inflammation or mild fibrosis was observed in the interstitium. Some tubular epithelial cells had enlarged or multiple nuclei. Tubular lumens were often dilated with proteinaceous casts.

Immunohistochemical examination revealed that homogeneous or fine fibrous deposits in the glomerular capillary walls and mesangial areas were intensely positive for type III collagen. On double immunostaining, these deposits were found to be localized in the subendothelial spaces and mesangial areas. Different localizations of type III and type IV collagen in the glomerular tufts were clearly observed (Fig. 1D). Localized deposition of fibronectin, IgG and IgM

were occasionally seen at the sites of capsular adhesion with segmental tuft sclerosis. IgA and C3 were not detected in the glomeruli. Few mesangial cells were positive for  $\alpha$ -SMA.

On electron microscopy, subendothelial spaces and mesangial areas were expanded with electron-lucent fibrils in U/L stained section (Fig. 2). T/U staining revealed that the fibrils had transverse bands with a periodicity of approximately 60 nm, indicative of collagen fibrils (Fig. 2, inset). These fibrils crossed randomly and appeared frayed or curved. Width of these fibrils was varied.

The clinical, histopathological, immunohistochemical and electron microscopic findings of the glomerular lesions in the present case were consistent with those of CFGN in both humans [2, 6] and animals [1, 5, 9, 11, 19]. On light microscopy, adhesion of glomerular tufts to the Bowman's capsules and cellular crescent formation indicated severe injury of the glomerular basement membrane (GBM) and podocytes, as seen in the Shiba Inu dog case [9]. Focal dep-

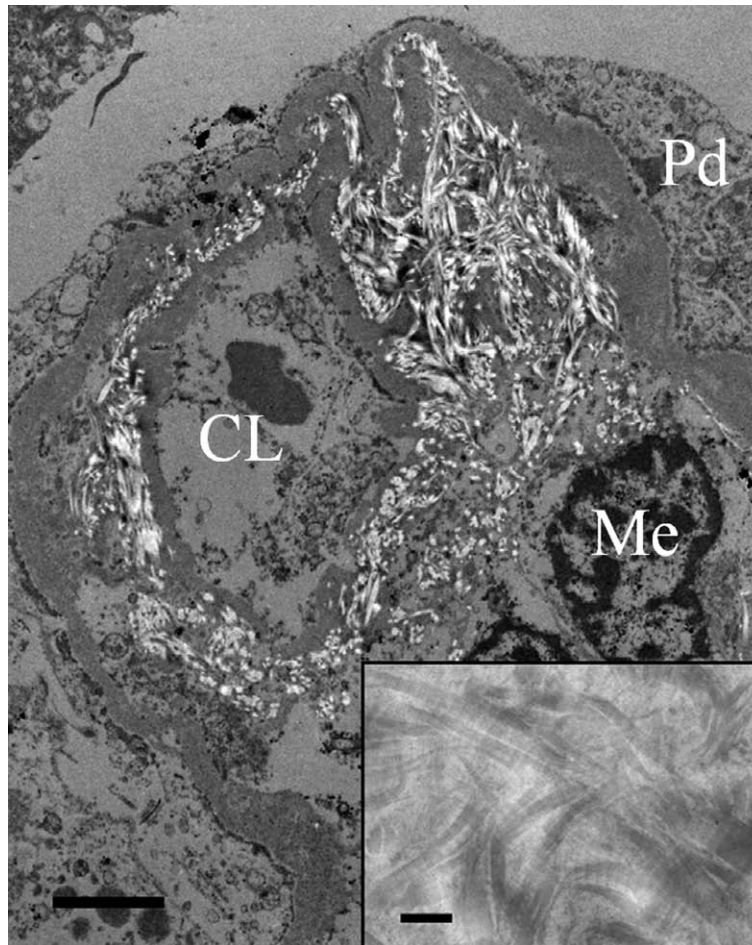


Fig. 2. Subendothelial spaces and mesangial areas were expanded with electron-lucent fibrils (CL, capillary lumen; Pd, podocyte; Me, mesangial cell). U/L staining. TEM.  $\times 2,500$ . Bar= $2\text{ }\mu\text{m}$ . (Inset) The fibrils crossed randomly and appeared frayed or curved and had transverse bands with a periodicity of 60 nm. Width of these fibrils was varied. T/U staining. TEM.  $\times 20,000$ . Bar= $200\text{ nm}$ .

osition of IgG and IgM may be non-specific and non-indicative of immunomediated processes in these glomerular lesions. In humans, focal segmental deposition of IgG, IgM and C3 may be observed, and this probably represents insudation of plasma proteins [2]. This indicates that abnormal type III collagen deposition induces injury of the GBM and podocytes. Electron microscopy also revealed an unusual array of accumulated fibrils with the characteristics of collagen fibrils as seen in other reports on CFGN [2, 18]. Clinically acute, progressive disease manifested by proteinuria and young age at onset might be comparable to the human childhood type of CFGN. Childhood CFGN is suspected to be an autosomal recessive disorder [7, 15]; however, we were unable to investigate family history to ascertain whether this was implied in the present case. Except for family history, clinical and histopathological findings of the present dog were similar to CFGN-like glomerulosclerosis in three Newfoundland dog littermates [10].

There remains some debate over whether type III collagen is formed within the glomeruli or whether it is derived from an extrarenal source. Although synthesis of type III collagen by glomerular epithelial cells has been demonstrated by *in situ* hybridization [13], it is not clear whether epithelial cells or endothelial cells are responsible for the production of type III collagen in subendothelial spaces. Using cell culture [4, 16] and *in situ* hybridization techniques [14, 17], type III collagen synthesis by mesangial cells has been also confirmed. In humans, mesangial cells positive for  $\alpha$ -SMA, which are thought to be activated cells, may produce type III collagen in CFGN [12]. Although few mesangial cells were positive for  $\alpha$ -SMA in the present case, it still possible that mesangial cells had been activated and produced type III collagen in the course of disease.

In humans, elevated serum procollagen type III peptide (PIIINP) is a good marker for CFGN [2]; however, serum levels of PIIINP were not examined in the present case. In a human CFGN autopsy case, massive accumulation of atypical collagen fibers was seen both in the kidneys and in systemic organs, including the spleen, liver, heart and thyroid glands [20]. In addition, high levels of serum PIIINP in human CFGN indicate the systemic nature of this condition. However, there have been no reports on systemic abnormalities associated with CFGN in animals, except in a congenital swine case [18]. In contrast, nail-patella syndrome (NPS) is a known systemic disease with abnormal type III collagen deposition. In NPS, type III collagen is distributed within a thickened GBM [3] and no specific glomerular changes are observed on light microscopy, in contrast to the diffuse glomerular changes seen in CFGN. Thus, we excluded a diagnosis of NPS based on these light and electron microscopic observations.

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## REFERENCES

1. Adachi, K., Mori, T., Ito, T., Fujii, E., Suzuki, S., Kawai, T. and Suzuki, M. 2005. Collagenofibrotic glomerulonephropathy in a cynomolgus macaque (*Macaca fascicularis*). *Vet. Pathol.* **42**: 669–674.
2. Alchi, B., Nishi, S., Narita, I. and Gejyo, F. 2007. Collagenofibrotic glomerulopathy: clinicopathologic overview of a rare glomerular disease. *Am. J. Kidney Dis.* **49**: 499–506.
3. Del Pozo, E. and Lapp, H. 1970. Ultrastructure of the kidney in the nephropathy of the nail-patella syndrome. *Am. J. Clin. Pathol.* **54**: 845–851.
4. Foidart, J. M., Foidart, J. B. and Mahieu, P. R. 1980. Synthesis of collagen and fibronectin by glomerular cells in culture. *Ren. Physiol.* **3**: 183–192.
5. Fujisawa-Imura, K., Takasu, N., Tsuchiya, N., Matsushima, S., Inagaki, H. and Torii, M. 2004. Spontaneous collagenofibrotic glomerulonephropathy in a young cynomolgus monkey. *J. Toxicol. Pathol.* **17**: 279–282.
6. Gubler, M. C. 2008. Inherited diseases of the glomerular basement membrane. *Nat. Clin. Pract. Nephrol.* **4**: 24–37.
7. Gubler, M. C., Dommergues, J. P., Foulard, M., Bensman, A., Leroy, J. P., Broyer, M. and Habib, R. 1993. Collagen type III glomerulopathy: a new type of hereditary nephropathy. *Pediatr. Nephrol.* **7**: 354–360.
8. Imbasciati, E., Gherardi, G., Morozumi, K., Gudat, F., Epper, R., Basler, V. and Mihatsch, M. J. 1991. Collagen type III glomerulopathy: a new idiopathic glomerular disease. *Am. J. Nephrol.* **11**: 422–429.
9. Kamiie, J., Yasuno, K., Ogihara, K., Nakamura, A., Tamahara, S., Fujino, Y., Ono, K. and Shirota, K. 2009. Collagenofibrotic glomerulonephropathy with fibronectin deposition in a dog. *Vet. Pathol.* **46**: 688–692.
10. Koeman, J. P., Biewenga, W. J. and Gruys, E. 1994. Proteinuria associated with glomerulosclerosis and glomerular collagen formation in three Newfoundland dog littermates. *Vet. Pathol.* **31**: 188–193.
11. Nakamura, S., Shibata, S., Shirota, K., Abe, K., Uetsuka, K., Nakayama, H., Goto, N. and Doi, K. 1996. Renal glomerular fibrosis in a cat. *Vet. Pathol.* **33**: 696–699.
12. Naruse, K., Ito, H., Moriki, T., Miyazaki, E., Hayashi, Y., Nakayama, H., Kiyoku, H., Hiroi, M., Kurashige, T. and Enzan, H. 1998. Mesangial cell activation in the collagenofibrotic glomerulonephropathy. Case report and review of the literature. *Virchows Arch.* **433**: 183–188.
13. Razaque, M. S. and Taguchi, T. 1996. Expression of type III collagen mRNA in renal biopsy specimens of patients with idiopathic membranous glomerulonephritis. *Clin. Mol. Pathol.* **49**: M40–M42.
14. Razaque, M. S., Koji, T., Taguchi, T., Harada, T. and Nakane, P. K. 1994. In situ localization of type III and type IV collagen-expressing cells in human diabetic nephropathy. *J. Pathol.* **174**: 131–138.
15. Salcedo, J. R. 1984. An autosomal recessive disorder with glomerular basement membrane abnormalities similar to those seen in the nail patella syndrome: report of a kindred. *Am. J. Med. Genet.* **19**: 579–584.
16. Scheinman, J. I., Brown, D. M. and Michael, A. F. 1978. Collagen synthesis by human glomerular cells in culture. *Biochim. Biophys. Acta* **542**: 128–136.
17. Scheinman, J. I., Tanaka, H., Haralson, M., Wang, S. L. and Brown, O. 1992. Specialized collagen mRNA and secreted collagens in human glomerular epithelial, mesangial, and tubular

- cells. *J. Am. Soc. Nephrol.* **2**: 1475–1483.
18. Shirota, K., Tanaka, N., Une, Y., Nomura, Y. and Kamei, M. 1995. Congenital renal glomerular fibrosis in a case of swine chondrodysplasia. *J. Vet. Med. Sci.* **57**: 151–154.
  19. Shirota, K., Masaki, T., Kitada, H., Yanagi, M., Ikeda, Y., Une, Y., Nomura, Y. and Jothy, S. 1995. Renal glomerular fibrosis in two pigs. *Vet. Pathol.* **32**: 236–241.
  20. Yasuda, T., Imai, H., Nakamoto, Y., Ohtani, H., Komatsuda, A., Wakui, H. and Miura, A. B. 1999. Collagenofibrotic glomerulopathy: a systemic disease. *Am. J. Kidney Dis.* **33**: 123–127.