Renal pathology of polycystic kidney disease and concurrent hereditary nephritis in Bull Terriers

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Objective To describe the renal lesions in Bull Terrier polycystic kidney disease (BTPKD), to confirm that the renal cysts in BTPKD arise from the nephron or collecting tubule, and to identify lesions consistent with concurrent BTPKD and Bull Terrier hereditary nephritis (BTHN).

Design Renal tissue from five Bull Terriers with BTPKD and eight control dogs was examined by light and transmission electron microscopy. Clinical data were collected from all dogs, and family history of BTPKD and BTHN for all Bull Terriers.

Results In BTPKD the renal cysts were lined by epithelial cells of nephron or collecting duct origin that were usually squamous or cuboidal, with few organelles. They had normal junctional complexes, and basal laminae of varying thicknesses. Glomeruli with small, atrophic tufts and dilated Bowman's capsules, tubular loss and dilation, and interstitial inflammation and fibrosis were common. Whereas the lesions seen in BTHN by light microscope were nonspecific, the presence of characteristic ultrastructural glomerular basement membrane (GMB) lesions and a family history of this disease indicated concurrent BTHN was likely in three of five cases of BTPKD.

Conclusion This paper provides evidence that renal cysts in BTPKD are of nephron or collecting duct origin. In addition, GBM lesions are described that strongly suggest that BTPKD and BTHN may occur simultaneously.
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ADPKD	Autosomal dominant polycystic kidney disease of humans
BTHN	Bull Terrier hereditary nephritis
BTPKD	Bull Terrier polycystic kidney disease
GBM	Glomerular basement membrane
H and E	Haematoxylin and eosin
PAS	Periodic acid Schiff
TEM	Transmission electron microscope
UPC	Urine protein to creatinine ratio

BTPKD is a recently recognised disease that, while not widespread, is common in some popular lines of Bull Terriers in Australia. It is characterised by an autosomal dominant mode of inheritance and bilateral renal cysts distributed throughout cortex and medulla.[1] Polycystic kidney disease with similar pathology and mode of inheritance occurs in humans,[2] cats [3] and rats,[4] and there are several targeted mutation models of ADPKD in mice.[5,6]

In contrast, BTHN is endemic in Australian Bull Terriers, consistent with comparatively long-term dissemination of the disease throughout the general population. The term hereditary nephritis describes a related group of inherited renal diseases, which recent studies in humans and dogs have shown to be caused by defective molecular structure of type IV collagen in the GBM.[7,8] BTHN also is inherited in an autosomal dominant manner,[9] and is characterised by glomerulopathy, progressive renal atrophy and renal failure at varying ages. Hereditary nephritis is also reported in Samoyeds (X-linked dominant), English Cocker Spaniels (autosomal recessive) and in a family of mixed breed dogs (X-linked dominant).[7,8,10] These canine hereditary nephritides are likely analogues of Alport's syndrome in humans. Targeted mutation models of Alport's syndrome have been created in mice.[11,12]

Histological features described in end-stage BTHN include the presence of microcystic glomeruli and cystic dilation of Bowman's capsule, persistence of foetal glomeruli in the adult, and the occurrence of juxtamedullary 'ectopic' glomeruli.[13,14] In dogs the kidney continues to develop postnatally,[15] and foetal glomeruli may be seen in normal dogs up to the age of 6 months. Ectopic glomeruli are believed to be embryological remnants, and occur in foetal, but not in adult kidneys.[16] Such glomeruli are mostly degenerate, occur at the margins of the pelvic septum and in connective tissue around the interlobar arteries, and are associated with long arteries that accompany large intrarenal vessels supplying both the pelvic plexus and renal medulla.[16] Other glomerular lesions described include diffuse hypercellularity, hypertrophy (tuft diameter > 170 μm), sclerosis, collapse and thickening of capillary loops, tuft atrophy and capsular fibrosis.[13,17] Radial fibrosis with infiltrates of macrophages, lymphocytes and plasma cells, and proteinaceous material within Bowman's space and tubules are also reported.[13,17] There is progressive loss of nephrons, and dilated tubules lined with hyperplastic epithelium develop with time.[13,17] However, none of these changes is specific to hereditary nephritis.[18,19] Definitive diagnosis requires demonstration by TEM of widespread thickening, splitting, and lamellation of the GBM with a 'basket weave' pattern.[7,10,20,21] Other ultrastructural features in BTHN include expansion and fusion of podocyte foot processes, subepithelial frilling, electron dense deposits between laminae, and subepithelial projections in the glomerular capillary basement membrane.[21]

The presence of two autosomal dominant renal diseases in Australian Bull Terriers raises the possibility of some individuals inheriting both, in which cases more severe renal disease might be expected, as well as an increased risk of producing offspring with at least one of the diseases. Veterinary clinicians and pathologists need to be able to identify cases with one or both these renal diseases for diagnostic and clinical management purposes, and to allow development of sound breeding strategies.

There are no published descriptions of the ultrastructure of lesions in BTPKD, or of lesions in cases with both BTPKD and BTHN. This study describes the gross, histological and ultrastructural features of the renal lesions in BTPKD, confirms that cysts in BTPKD arise from the nephron or collecting tubule,

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and identifies pathological features consistent with concurrent BTHN and BTPKD.

Materials and methods

Animals

Five Bull Terriers (cases 1-5) with BTPKD were selected for study (Table 1) and pedigrees obtained. All were between 3.5 and 28 months of age, female, and regarded as normal by their owners. All were euthanased within one week of renal ultrasonographic diagnosis of BTPKD. Blood pressure was not measured in any of the dogs. Clinical data on the parents were also collected.

Four healthy, crossbred, control dogs were selected. They were phenotypically not Bull Terriers and so not likely to have BTHN: one female and one male, 1 to 2 years of age, weighing between 15 and 20 kg, and one male and one female, 3 to 4 months of age, weighing between 6 and 8 kg.

Four healthy control Bull Terriers were selected: three males: 5, 12 and 18 months old, and one female, 5 months old. Clinical data on the parents of these animals were collected to establish the absence of family history of BTPKD and BTHN.

Diagnostic criteria

The diagnosis of BTPKD required ultrasonographic or necropsy demonstration of at least three cysts distributed between both kidneys, together with the occurrence of other affected first-degree family members, including parents, siblings or offspring from a mating to an unaffected animal.[1]

The diagnosis of BTHN is based on three criteria: persistent proteinuria of glomerular origin, a family history of affected first-degree relatives, and the presence of characteristic GBM ultrastructural changes.[13] Proteinuria of glomerular origin is indicated by demonstrating a UPC ratio greater than 0.3, with no significant urinary sediment, in an otherwise normal animal.[1,22]

Cardiac valvular disease was assessed by auscultation for murmurs, and by pathologic evaluation and was graded according to standard criteria.[23,24]

Clinical examination

Clinical data were collected to detect other renal or cardiac diseases that could influence interpretation of renal lesions. Because of the endemic nature of BTHN, particular emphasis was placed on data relating to the diagnosis of this disease.

A midstream urine sample was collected from cases 1 and 2 on the day before euthanasia, and a cystocentesis sample from cases 3 and 5 about one week before euthanasia. No urine was collected from case 4. All control animals had voided urine samples collected on the day of euthanasia. All samples were kept cool and examined within 36 h of collection.

All urinalyses were performed by the same person, using Ames multiple reagent strips (Bayer Diagnostics), a haemocytometer for microscopic examination of sediment, a Reichert veterinary refractometer for urine specific gravity determination (Cambridge Instruments) and a digital pH meter. The creatinine concentration was measured using the picric acid method and the Jaffe reaction with a creatinine reagent kit (Trace Scientific), and protein was measured using the pyrogallol method and urinary protein kit (Randox Laboratories) on a Cobas Mira (Roche Diagnostics Systems) wet chemistry analyser. After measuring urine protein and creatinine values, the UPC was calculated.

Table 1. Protein: creatinine ratio and cell content in single urine sample from dogs with BTPKD.

Case No	Age (months)	UPC[ab]	Urine cells[c] (ca/mL)
1	3.5	0.36v	10, 503, 3
2	7	0.08v	20, 203, 28
3	4	0.38c	194, 250, 0, cocci
4	28	not available	not available
5	20	0.28c	1517, 25, 0

[a]UPC urine protein: creatinine ratio
[b]method of urine collection: v voided, c cystocentesis
[c]numbers left to right are red blood cells, leucocytes and epithelial cells, for reference values for UPC and urinary cell numbers see reference[1]

Table 2. Glomerular basement membrane width in control (normal) dogs, calculated from five measurements per glomerulus on five glomeruli per dog.

Breed	Number	GBM width (nm)		
		mean	SD	Prediction interval
Crossbred	2 pups, 2 adults	170	20	130-210
Bull Terrier	2 pups, 2 adults	180	30	120-240

All control animals had extended haematological and biochemical renal profiles performed.

Since heart disease may affect the GBM,[25] all cases were clinically evaluated by auscultation. Cases 1 and 2 and all controls were auscultated unsedated, and cases 3 to 5 after sedation with 2 mg/kg acepromazine per os. Cases 1 and 2 and all Bull Terrier controls were on heartworm prophylaxis.

Necropsy

All Bull Terriers (cases 1-5) with BTPKD were euthanased by barbiturate overdose within one week of renal ultrasonographic diagnosis of the disease. In cases 1 and 2 a full necropsy, excepting examination of the brain and spinal cord, was performed, with renal and cardiac tissue collected. In cases 3, 4, and 5 only formalin-fixed kidneys and hearts were available for study.

All control animals were euthanased by barbiturate overdose, a full necropsy was performed as above, and renal and cardiac tissue collected.

Light microscopy

Renal tissue was fixed by immersion in 10% neutral buffered formalin and processed routinely for paraffin embedding. Sections of 5 μm thickness were cut and stained with H and E, Jones methamine silver, Massons trichrome, and PAS.

Each dog had more that 30 glomeruli evaluated in H and E sections. Semi-quantitative estimates of glomerular tuft size were made using a 125 μm graticule with a x 20 objective. A further minimum of ten glomeruli from each dog was examined in 1 mm epon embedded sections stained with toluidine blue. Five of these glomeruli were then examined ultrastructurally.

Transmission electron microscopy

For TEM there was some variation in tissue sampling between animals, as tissues were processed in different laboratories. In cases 1 and 2 and non-Bull Terrier control animals, 1 mm cubes of renal tissue were collected within 30 min of

Table 3. Evidence of BTHN in cases of BTPKD in this study.

	Case 1	Case 2	Case 3	Case 4	Case 5
Foetal glomeruli	Many, some cystic	Many, some cystic	Many in a minority of sections	1 per section, 1 cystic	Many in a minority of sections
Ectopic glomeruli	Few	Few	1 as total count	1 as total count	Few
GBM[a]	Some splitting; 1 GBM lesion per capillary loop in glomerulus	Some splitting; few severe lesions per glomerulus	Occasional bilaminar splitting	Moderate amount of GBM with bilaminar splitting	Few severe lesions per glomerulus
Sire diagnosis[b]	BTPKD UPC > 0.3, family history of BTHN	No BTPKD UPC < 0.3 at 5Y	BTPKD[b] BTHN	BTPKD family history of BTHN	BTPKD[b] BTHN
Dam diagnosis	No BTPKD UPC < 0.3 at 2Y, family history of BTHN	BTPKD BTHN by LM, family history of BTHN	No BTPKD UPC < 0.3 at 7Y	BTPKD UPC > 0.3, family history of BTHN	No BTPKD UPC < 0.3 at 7Y
Evidence for BTHN	LM strong, TEM strong	LM strong, TEM suspicious	LM low, TEM nil	LM low, TEM nilL	LM strong, TEM suspicious

[a]Lesions characteristic of BTHN, seen by TEM
[b]Sire X had BTPKD and was diagnosed with BTHN based on having an offspring with BTHN when mated to an animal free of BTPKD and with a normal UPC at the age of seven years. Sire X was a parent of cases 3 and 5 and a paternal grandparent of case 1
Y = years old, LM = light microscopy, TEM = transmission electron microscopy

euthanasia, and fixed overnight at 4°C in 3% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in acetone and embedded in Spurr's resin. Sections of 1 μm thickness were cut using a glass knife, stained with toluidine blue and examined by light microscope. The region of interest was trimmed, cut into 80 to 90 nm sections with a diamond knife, retrieved onto copper grids, stained with uranyl acetate and lead citrate, and examined by TEM.

Renal tissue from cases 3, 4, and 5 was initially formalin-fixed. This tissue was cut into 1 mm cubes and washed in Sorensen's phosphate buffer for 15 min 10 or 11 times, with a final overnight wash to remove the formaldehyde. Following 3 h fixation at 4°C in 2.5% glutaraldehyde, the tissue was immersed in 1% Dalton's chrome osmic acid for 30 min, dehydrated in ethanol, and embedded in Epon. Sections for TEM were then prepared as described above.

Renal tissue from the four Bull Terrier control animals was collected within 30 min of euthanasia, cut into 1 mm cubes, fixed in 3% glutaraldehyde at 4°C, post-fixed in 1% osmium tetroxide, dehydrated in ethanol and processed as for the formalin fixed tissue.

Measurements of cyst basement membrane from a minimum of five cysts were taken from each of cases 1, 2 and 3. The width of cyst basement membrane was measured on electron micrographs taken at x 4800 magnification and enlarged by a factor of three on the print, using the printed scale on each photographic print and a digital vernier caliper with a resolution of 0.01 mm. Each case and control animal had five measurements taken from each of five glomeruli, and the mean, standard deviation and 95% prediction intervals for GBM width were calculated.

Immunohistochemistry

Immunohistochemical staining was performed on paraffin-embedded, formalin-fixed, renal tissue. Expression of coagulation factor VIII-related antigen was detected according to the manufacturer's protocol using factor VIII alkaline phosphatase (code A082; Dako LASB kit, KO674) and fast red as a chromogen. Expression of cytokeratin was detected using wide spectrum, polyclonal keratin antibody (Z0622), which has been reported to react with cytokeratin types 1, 5, 6, 8, 13, and 16. The protocol used was as described in the Dako LSAB2 Immunoperoxidase Peroxidase (K0675) kit, with 3-amino-9-ethylcarbazole used as a chromogen. Positive staining was assessed by comparing staining intensity with positive and negative controls. In addition, cytokeratin staining was graded by comparing staining intensity of cyst epithelia with intensely stained pelvic epithelium, the latter arbitrarily assigned a value of 4+.

Results

Controls

Clinical — All non-Bull Terrier and Bull Terrier controls had normal extended haematological and biochemical renal profiles, UPC ≤ 0.3 and normal urinalyses from voided midstream urine samples.[1] Both parents of each adult control Bull Terrier had UPC ≤ 0.3 at ages of 2.5, 3.5 or 5 years, as did both parents of the control Bull Terrier pups at 1 and 4 years of age.

No control had a cardiac murmur.

Necropsy — All were grossly normal except one 12-month-old adult Bull Terrier that had three simple cysts in one renal cortex. None had adult *Dirofilaria immitis* infection.

Light microscopy — Renal histology was normal except for the presence in non-Bull Terrier controls of foetal glomeruli in one adult and both pups, and ectopic glomeruli in the second adult and both pups. Foetal glomeruli were also present in kidneys from all control Bull Terriers, and ectopic glomeruli in both pups.

Transmission electron microscopy — Renal ultrastructure was essentially normal in all control animals except that focal bilaminar splitting of the lamina densa of the GBM was observed occasionally in one adult and one pup non-Bull Terrier, and both adult Bull Terriers and one pup.

GMB thickness varied between 120 and 240 nm, and up to 380 nm in some cases where bilaminar splitting occurred (Table 2).

BTPKD group

Clinical — Two of the four cases for which urinalysis results were available had an increased UPC, but as all had increased urinary sediment this may have been due to urinary or repro-

ductive tract inflammation, and unrelated to BTHN or BTPKD (Table 1). Hence, UPC was not diagnostically useful in any of these cases.

Pedigree records showed all five cases were descended from a single affected ancestor. In addition, three were descended from one animal, sire X, which was a parent of cases 3 and 5 and a paternal grandparent of case 1.

Pedigree analysis also showed all cases of BTPKD had a family history of BTHN, with one or both parents of each case likely to have had BTHN. This likelihood was based on family history of the disease, including disease in offspring produced from matings with normal animals, on UPC values, or on light microscopic examination of renal tissue (Table 3).

Sire X had BTPKD and was diagnosed with concurrent BTHN on the basis of having produced an offspring with BTHN when mated to an animal free of BTPKD which had a normal UPC at the age of seven years (Table 3). Sire X was the sire of two cases (3 and 5), and the paternal sire of case 1. The sire of case 1 had an elevated UPC with normal urinary sediment [1] and was therefore likely to have had BTHN. Case 2 had one parent diagnosed with BTHN on the basis of light microscopic findings and a strong family history of the disease. Both parents of case 4 had BTPKD, one had an elevated UPC with normal urinary sediment, while the other parent had a strong family history of BTHN, although no urinalysis result was available.

None of the cases were in congestive heart failure. However, case 4 had a soft murmur typical of mitral regurgitation.[23]

Necropsy — Necropsy findings on cases 1 and 2 were normal apart from renal and cardiac pathology.

Both kidneys from all five cases with BTPKD were either normal in size or mildly enlarged. Some were irregular in shape. Cysts were present in cortex and medulla of both kidneys of all cases, but were often asymmetrically distributed and concentrated at the corticomedullary junction. Cysts were usually spherical, up to several centimetres in diameter, and some were multilocular (Figure 1). They generally contained fluid that was clear, straw-coloured, serosanguinous, bloody, or brown. Some contained pus and necrotic debris. Focal capsular adhesions were seen occasionally, usually associated with irregular, depressed areas, and sometimes corresponding with irregular thinning of cortex.

In addition to polycystic kidneys, case 3 had one kidney with a thickened capsule and an irregularly eroded cortex involving half to a third of one pole.

Cardiac disease observed grossly included mild endocardiosis of the aortic valve in case 2, mitral valve in case 3, aortic and mitral valves in case 5, and aortic, mitral and tricuspid valves in cases 1 and 4.[24] No adult *D immitis* were present.

Light microscopy — Cysts were usually lined by a single layer of squamous or low cuboidal epithelial cells (Figure 2), though occasionally multilayered, transitional epithelia were observed. Most cysts appeared empty in histological section, but a few contained sloughed epithelial cells, red blood cells, macrophages and neutrophils. Some cysts also contained proteinaceous material with cholesterol clefts, indicating earlier haemorrhage. Staining with Jones methamine silver, PAS and Massons trichrome showed that the epithelium was supported by a basement membrane of variable thickness (Figure 3). In addition to overt cystic structures, many tubules and some glomerular capsules were dilated to varying degrees, and some tubules were lined by stratified epithelium. Interstitial lymphocytic-plasma-

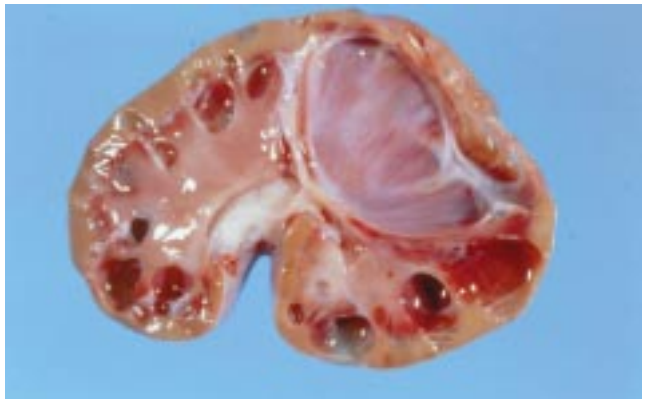

Figure 1. Kidney from a Bull Terrier with BTPKD showing cortical and medullary cysts.

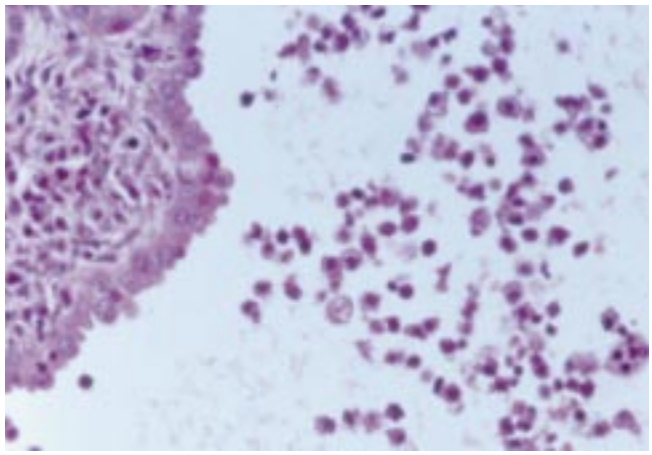

Figure 2. BTPKD renal cyst lined by cuboidal epithelial cells, and containing sloughed, degenerate cells and macrophages. Haematoxylin and eosin, x 80.

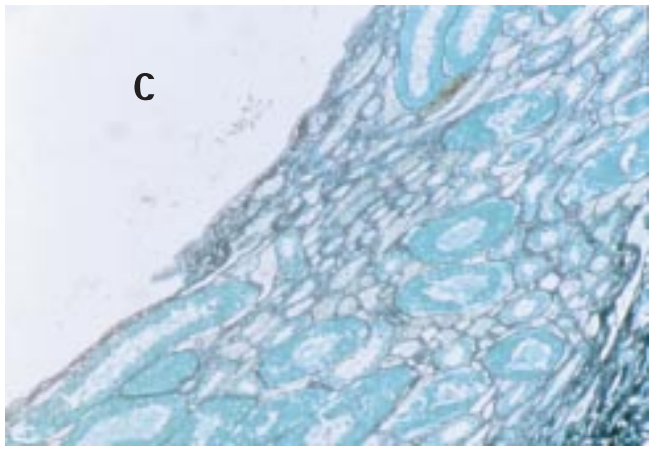

Figure 3. BTPKD cyst wall showing a silver positive structure consistent with a basal lamina. C = Crypt lumen Jones methamine silver, x 25.

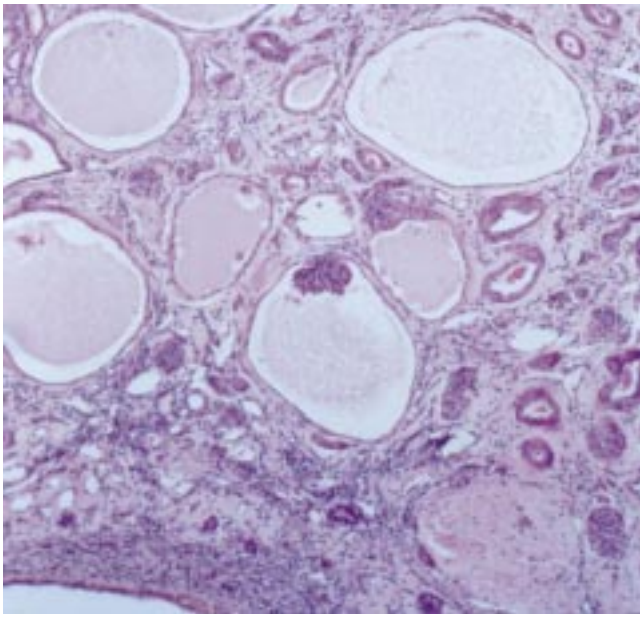

Figure 4. BTPKD renal parenchyma showing interstitial fibrosis, inflammation, lack of tubules, dilated Bowman's capsules and primitive, small (foetal) glomerular tufts. H and E, x 40.

cytic inflammation and fibrosis were common, and occurred particularly in proximity to cysts (Figure 4). Occasional focal infiltrates of neutrophils, tubular collapse and nephron loss were also evident.

A number of glomerular abnormalities that have been reported to often occur together in BTHN,[13,14] were found in cases 1, 2 and 5 (Table 3). Thus, foetal glomeruli (Figures 4, 5) were numerous, some ectopic glomeruli were present, and numerous glomeruli with small, consolidated tufts, dilated or cystic Bowman's capsules sometimes filled with eosinophilic, proteinaceous material, and sometimes having hyperplasia of the parietal epithelial cells were present. These cystic atrophic glomeruli were present in areas of interstitial inflammation and fibrosis, and may have resulted from these changes. Various combinations of glomerular hypercellularity, hypertrophy, hyperlobulation and diffuse mesangial sclerosis were evident in all five cases.

Based on renal histology, cases 1, 2 and 5 were considered likely to have concurrent BTHN, whereas cases 3 and 4 were considered to have no firm histological indicators of BTHN, as the foetal glomeruli in case 3 were probably a reflection of its young age.

In case 3, one kidney had a locally extensive area of subcapsular granulation tissue with organising fibrin on the overlying capsular surface. This lesion was interpreted to be an infected, ruptured cyst. In case 2 there was a focal area of loose myxoid interstitial connective tissue, consistent with that described in renal dysplasia.[26]

In all five cases many of the small arterioles associated with glomeruli and interpreted to largely represent afferent arterioles, were thickened by medial and adventitial proliferation, producing an 'onion skin' pattern of hypertrophy.

Transmission electron microscopy — Renal cysts were lined by squamous or cuboidal epithelial cells, the great majority of which had no specific features to indicate which part of the nephron or collecting tubule they may have arisen from.

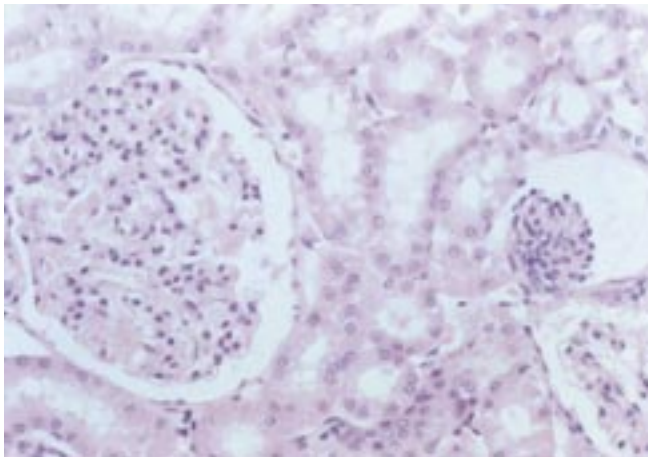

Figure 5. Normal mature glomerulus on left and foetal glomerulus on right. H and E, x 80.

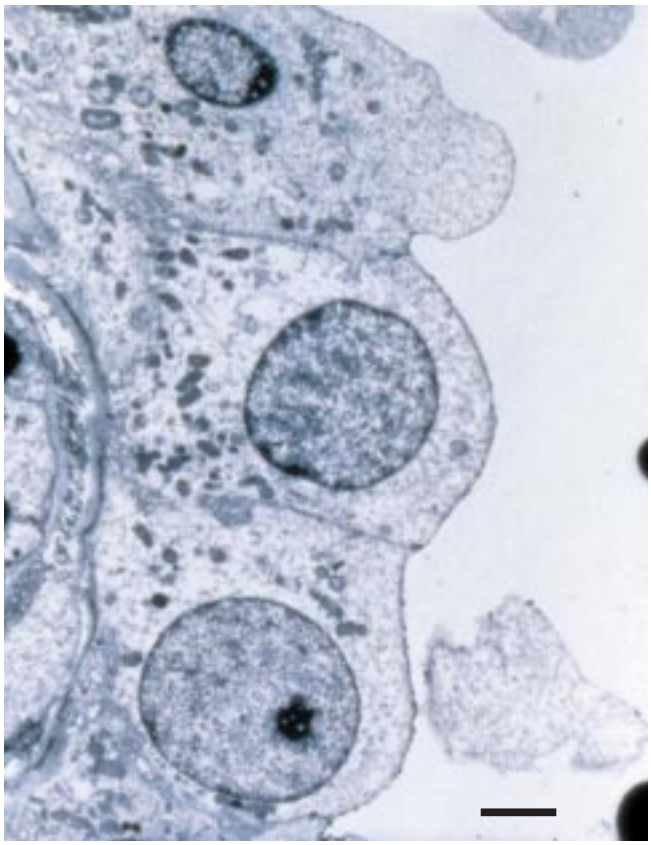

Figure 6. Cuboidal cyst epithelium lacking microvilli and containing few organelles, but having normal cell junctions. TEM, bar = 2 μm.

Whereas residual brush borders on cells in one cyst indicated proximal tubule as the likely origin, most had no, or only vestigial, microvilli (Figures 6, 7). These cells had few of the specialised features of renal epithelium, and often a paucity of organelles. Some cells had an extremely low profile. Cells were in close apposition and normal inter-cellular junctional complexes were present (Figure 7). Most had no basal or lateral infoldings, but some had outward foldings of the basal cell surface enclosing transport vesicles.

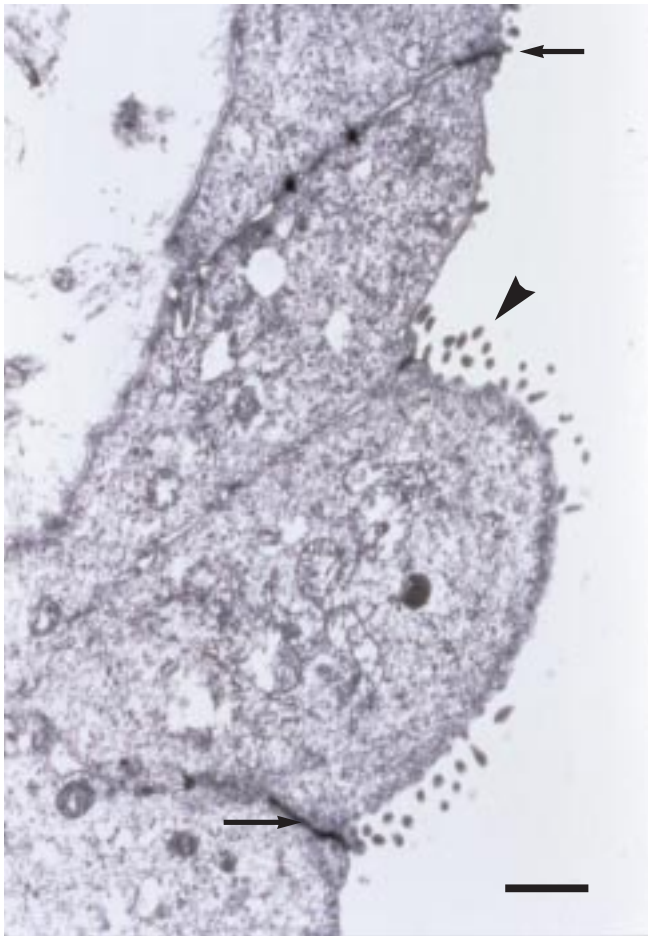

Figure 7. Cuboidal cyst epithelium with microvilli (arrowhead), few organelles and membrane systems, and normal cell junctions (arrows). Some damage to intracellular structures is present and is probably post-mortem artefact. TEM, bar = 1 μm.

The basal lamina of cysts varied between 15 and 550 nm in thickness, and had a variable amount of collagen immediately beneath. While interstitial collagen was minimal in control animals, in those with BTPKD significant subcystic deposits were sometimes present, with disorderly arrangement of fibrils, and activated fibroblasts.

In cases 1, 2 and 5 the diffuse GMB lesions reported as characteristic of BTHN were either focally severe, or extensive (Table 3). These lesions included thickening, splitting, and lamellation of the GBM, fusion of epithelial foot processes, occasional dense deposits, subepithelial frilling and subepithelial projections from the glomerular capillary basement membrane (Figures 8, 9).[13,21] The thickness of the GBM commonly was up to 0.5 μm, and in some lesions to 2 μm. Such lesions were not found in cases 3 or 4, although they did have some bilaminar GBM splitting.

Immunohistochemistry — Staining for factor VIII-related antigen in case 2 was absent in cyst lining cells (Figure 10), consistent with these being non-endothelial in nature. Staining for cytokeratin in case 2 was strong (4+) in pelvic epithelium, weak (1+) in proximal and distal tubules (2+), and absent in Bowman's capsule. Whereas some squamous and cuboidal cells stained strongly (Figure 11), others in the same cyst, with apparently identical morphology, did not stain and in some cysts no cells stained positively.

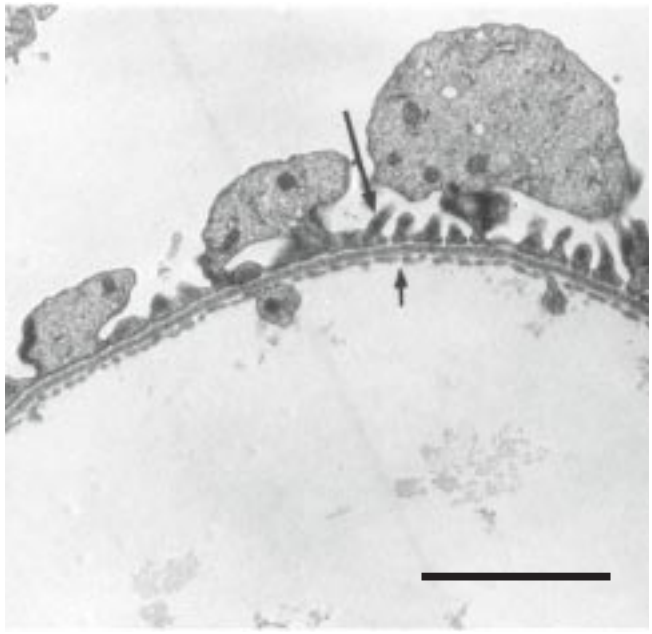

Figure 8. Normal trilaminar glomerular basement membrane. Visceral epithelial cells and their foot processes (long arrow) cover the outer urinary surface of the GBM. Endothelial cells stretch over the opposite inner surface of the GBM, with numerous pores (short arrow) present. The GBM itself consists of the central broad lamina densa, and two electron light layers, the lamina rara externa, and the lamina rara interna. TEM, bar = 2 μm.

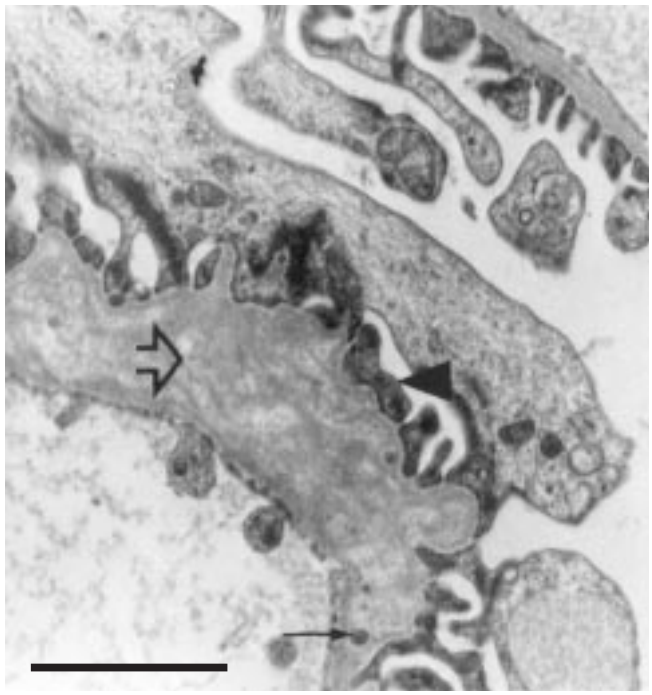

Figure 9. GBM thickening, lamellation (hollow arrow), electron dense particles (thin arrow) and epithelial foot process fusion (arrowhead) from a dog with BTPKD and BTHN. TEM, bar = 2 μm.

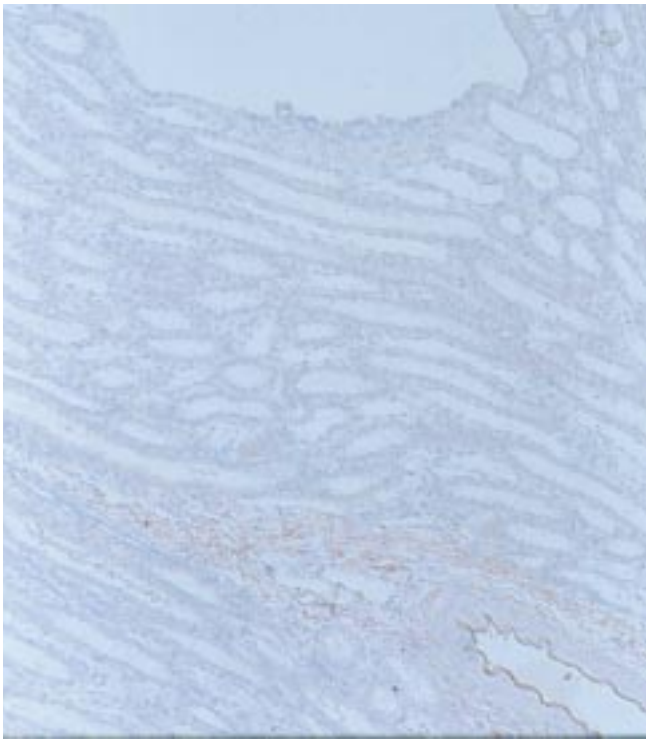

Figure 10. BTPKD renal section showing absence of factor VIII-related antigen staining in cyst-lining cells, and positive staining (red) in a nearby vessel. Immumohistochemical stain, x 25.

Figure 11. Same BTPKD renal section showing positive cytokeratin staining (red) of cyst-lining cells and negative staining in a nearby vessel. Immunohistochemical stain, x 25.

Discussion

Renal pathology in BTPKD

BTPKD is inherited in an autosomal dominant manner, and is characterised by bilateral renal cysts in cortex and medulla, and a family history of the disease.[1] Histology, immunohistochemistry and TEM have confirmed an epithelial and non-endothelial origin for at least some of the cyst-lining cells. However, determining the nephron segment of origin of cysts proved impossible using morphological criteria alone, due to the simplified appearance of most lining cells. This inability to determine nephron segment of origin also occurs commonly in ADPKD and polycystic kidney disease in cats, rats and genetically modified mice, because cyst-lining cells are often organelle poor, with retention of morphological features of the segment of origin only likely early in the disease.[4,6,27,28]

In BTPKD, both the morphology and immunohistochemical cytokeratin staining of lining cells varied in some cysts. This may indicate loss or nondevelopment of phenotypic features and epithelial antigens, modulation of cytokeratin expression, or coalescence of cysts. Similar variation in phenotype has been reported in human ADPKD.[29]

In BTPKD, the basement membranes of the cysts varied between 15 and 550 nm in thickness. Tubular basement membrane (TBM) thickness has been reported to be 129 $\pm$ SD 62 nm in normal pups, and 467 $\pm$ SD 127 nm in adults.[21] These large standard deviations indicate wide variations in TBM thickness, likely related to tubule type. Hence, as nephron segment of origin could not be determined in renal cysts in BTPKD, it was not possible to judge whether the basal lamina was thicker or thinner than normal.

In polycystic kidney disease in other species the cyst basement membrane appearance may differ from that in Bull Terriers. In ADPKD it varies from a thin, electron dense layer resembling that surrounding normal tubules, to an extensively thickened, and sometimes variably dense, laminated or cribriform structure believed to be unrelated to the segment of origin.[27] In rats the membrane may be thickened and split,[4] but it is normal in cats.[28]

In addition to cysts, cases of BTPKD in this study also showed glomerular abnormalities, including foetal and ectopic development, hypercellularity and hypertrophy, focal hyperlobulation and mesangial sclerosis. While these lesions in cases 1, 2 and 5 may have been due to concurrent BTHN, they were also present in cases 3 and 4, which did not have the characteristic lesions of BTHN. This may indicate that glomerular abnormalities occur in BTPKD.

The occurrence of glomerular lesions in BTPKD is reinforced by their occurrence in the similar disease of humans. In end stage ADPKD, glomerular lesions include tuft atrophy, global (but not segmental) sclerosis, hypertrophy and increased mesangial matrix.[27,30,31] Similarly, an increase in glomerular mesangium, a segmental increase in thickness of the glomerular capillary walls, and many sclerotic glomeruli have been described in a cat with end stage polycystic kidney disease,[32] and in rat polycystic kidney disease.[33]

Renal histology in the diagnosis of BTHN

Although histological lesions have been described in uraemic dogs with BTHN,[13,17] many of the changes are likely to be secondary, compensatory or non-specific, and were variable in

severity and regional distribution both between and within the kidneys. Adult Bull Terriers and other breeds with neither family history, nor clinical or ultrastructural evidence of BTHN, may have persistent foetal and ectopic glomeruli. This suggests that persistence of these structures in adults is not confined to either BTPKD or BTHN, and is not specific to the latter. These findings reinforce the absolute requirement for ultrastructural examination of the GBM for the diagnosis of BTHN as it offers what is the closest to pathognomonic lesions. This is in agreement with other studies, which report that lesions detectable by light microscopy in canine hereditary nephritis and in human Alport's syndrome may be absent, nonspecific or diagnostically unreliable in some cases.[18,19] Despite this, light microscopy does have a place in diagnosis, and may allow exclusion of other nephropathies.[18]

GBM lesions in BTPKD and BTHN

The concurrence of BTHN and BTPKD in the same animal is significant as it may lead to more severe renal disease than occurs with either disease alone. The risk of the progeny having at least one of these defects is obviously also increased. It is desirable therefore to be able to recognise lesions that are specific to either disease and which are shared by both, and possibly other diseases. The most promising tissue candidate for this is the GBM.

Ultrastructural GBM lesions including focal or diffuse GBM thickening, splitting, and lamellation were present in three of our cases of BTPKD, with a further two cases showing only focal bilaminar splitting.

Interpretation of these GBM lesions as being caused by BTPKD may be difficult if the concurrent presence of BTHN also has to be considered. While it is ideal to have all three diagnostic criteria of BTHN established to confirm the presence of this disease, this may not always be possible, as, for example, in some of our cases, where measurement of the UPC data were consistent with urinary tract inflammation and/or haemorrhage, rather than glomerulopathy. In addition, proteinuria has been reported in cases of BTPKD,[1,34] and may be part of the clinical spectrum of this disease rather than due to concurrent BTHN. Proteinuria also occurs in ADPKD,[2] and polycystic kidney disease in cats,[35] and rats.[4]

Ultrastructural lesions of the GBM, including widespread thickening, splitting, lamellation and basket weave pattern, are considered characteristic of hereditary nephritides in Bull Terriers, Samoyeds, English Cocker Spaniels and a family of mixed-breed dogs.[7,13,20,26] Focal lesions have also been described, particularly early in the disease process. In Alport's syndrome, a similar range of ultrastructural lesions of the GBM is considered to be pathognomonic.[18]

Splitting and duplication of the GBM occurs in the early stages of ADPKD,[37] and homogeneous segmental thickening and wrinkling in the late stages.[30] GBM thickening has been reported in polycystic kidney disease in rats.[33] Dogs with BTPKD in this study had multifocal bilaminar splitting in excess of the 1.5% of GMB reported in normal dogs,[10] but not the characteristic GBM lesions of BTHN. In hereditary nephritis bilaminar splitting of the GBM may precede development of the basket weave pattern.[11,19,20,38] However, the dogs in this study did not show other early GBM ultrastructural changes that occur in BTHN including subepithelial frilling, vacuolation, epithelial foot process effacement and expansion of the mesangial matrix, which have been reported to be the predominant lesions.[21] Hence, splitting and duplication of the GBM in BTPKD appears likely.

To further complicate interpretation of glomerular and GBM lesions in BTPKD, congestive heart failure has been reported to cause histological and ultrastructural glomerular lesions.[25] However, whereas cardiac disease was detected in all cases of BTPKD in this study, none showed clinical signs of congestive heart failure. It is thus concluded that the histological lesions and the ultrastructural changes in the GBM were not likely to have been secondary to heart disease.

Hence, while glomerular ultrastructure is likely to be the most useful diagnostic feature of concurrent BTPKD and BTHN, definitive diagnosis may require test matings, or ideally the development of a molecular diagnostic test for both diseases.

Renal Failure

Eventual renal failure in ADPKD and polycystic kidney disease in rats and cats is thought to follow progressive interstitial inflammation, fibrosis and matrix accumulation.[4,27,28] Glomerular lesions and tubular loss may also contribute to the development of renal failure.[27,32,33]

In this and previous studies of BTPKD, dogs aged from 7 weeks to > 6 years did not show signs of renal failure, however few were tested for azotaemia.[1,34] Based on similar histopathological findings in BTPKD and polycystic kidney disease in other species, the few cysts and abundant normal renal parenchyma found in most reported BTPKD dogs, progression of renal pathology and eventual renal failure would be expected in BTPKD, probably in middle to old age.

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BOOK REVIEW

Introduction to Animal Technology, *2nd edn. Barnett SW, Iowa State University Press, Ames, 2001, 112 pages. Price US$39.99. ISBN 0632 05594 4.*

This handbook has been written for novice animal technology students, and is published by the Institute for Animal Technology – the organisation responsible for establishing the standards and credentials for animal technicians in the UK.

The contents address the needs of staff working in laboratory animal houses with sections covering animal health, breeding, environmental control of animal houses, identification, hygiene, safety, as well as animal husbandry. The content is organised in terms of topics rather than species, this is a disadvantage to the reader wanting to know about a particular species.

The chapter on legislation pertaining to the use of animals for scientific procedures reflects the British Act and although there are similarities in the intent the procedures and terminology are different from comparable legislation in Australia. Since Australian animal welfare legislation is under state juristriction this makes any information potentially confusing for students.

Some of the physical techniques described for euthanasia would not be acceptable practice in Australian animal houses.

Hamsters, rabbits, rats, mouse, ferret, Guinea pigs, cats, dogs, and birds rate a mention – but this is not a manual for anyone requiring information sufficient to enable a novice to care for a species. Considering the most common animal used in research is the mouse there is surprisingly little information on this species.

The glossary and the appendix 'Summary of the breeding of laboratory animals' is a useful addition.

Environmental enrichment is restricted to nesting materials – no mention of social groupings, feeding practices or management systems, which are important topics in the field of current laboratory animal science and welfare.

Excellent photographs are provided to assist one picking up the animal and working out if it is a boy or girl.

This book is for the animal attendant – however most educational insititutions provide more relevant notes/CD's or online manuals to cover the material in a manner more suited to Australian animal house workers, for a fraction of the price of this publication. The locally produced (University of Melbourne) CD and videos, *Careful how you hold me,* is more relevant and useful for teaching purposes in Australia.

For those wanting a useful laboratory animal reference it is better to put the money to a UFAW manual!

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