Ultrastructural Appearance of Renal and Other Basement Membranes in the Bull Terrier Model of Autosomal Dominant Hereditary Nephritis

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• **Bull terrier hereditary nephritis may represent a model for autosomal dominant Alport's syndrome because affected dogs have the typically lamellated glomerular basement membrane (GBM) and father-to-son disease transmission occurs. This study examined the ultrastructural appearance of the renal and extrarenal basement membranes and their composition in affected Bull terriers. Affected stillborn animals and puppies had subepithelial frilling and vacuolation of the GBM. In adult dogs, lamellation was common, and subepithelial frilling and vacuolation were less prominent. Foot-process effacement and mesangial matrix expansion occurred frequently. Basement membranes in the glomeruli, tubules, and Bowman's capsule were significantly thickened and often mineralized. Immunohistochemical examination showed α1(IV) and α2(IV) collagen chains in all renal basement membranes; α3(IV), α4(IV), and α5(IV) chains in the GBM, distal tubular basement membrane, and Bowman's capsule; and the α6(IV) chain in Bowman's capsule. Conversely, the basement membranes from the affected Bull terrier cornea, lens capsule, retina, skin, lung, and muscle had a normal ultrastructural appearance and were not thickened compared with membranes in normal age-matched dogs. The distribution of basement membrane abnormalities in Bull terrier hereditary nephritis may occur because the defective protein is present exclusively or more abundantly in the kidney and is structurally more important in the kidney or because of local intrarenal stresses.**

INDEX WORDS: Alport's syndrome; collagen type IV; glomerular basement membrane (GBM); Goodpasture antigen.

MOST PATIENTS with Alport's syndrome have the X-linked form in which affected males have hematuria and subsequently develop renal failure, deafness, and often anterior lenticonus and retinopathy.[1-3] In X-linked disease, all mutations affect the COL4A5 gene,[4,5] resulting in the loss of the α5(IV) collagen chain, together with the α3(IV) and α4(IV) chains from the glomerular (GBM) and other affected basement membranes.[6] Autosomal recessive Alport's syndrome is less common, and mutations affect the COL4A3/COL4A4 genes[7] that encode the α3(IV) and α4(IV) chains. These, together with the α5(IV) chain, are usually absent from the GBM of affected patients.[8]

Autosomal dominant disease is rare. The clinical features differ from the phenotype seen in X-linked and autosomal recessive Alport's syndrome. Some affected individuals have hematuria without renal failure and eye abnormalities do not occur, and nearly all individuals have large platelets, low platelet counts, and neutrophil inclusions.[9-13] The α3(IV), α4(IV), and α5(IV) collagen chains are present in affected GBM,[14] and autosomal dominant Alport's syndrome is genetically heterogeneous, with linkage shown to the locus for autosomal recessive Alport's syndrome (COL4A3/COL4A4) in one family[15] and to a novel locus in others.[16]

The diagnosis of Alport's syndrome usually depends on the presence of the typical clinical features, together with a family history of the disease or demonstration of a lamellated GBM on ultrastructural examination of the renal biopsy specimen.[17-20] The initial abnormality seen in the GBM of children is a focal or diffuse attenuation, which may persist in females as the

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only abnormality throughout life. In adult men, the GBM is usually thickened threefold to fivefold and split into two or more lamellae that branch and rejoin to form a basket weave with irregular spaces, sometimes containing electron-dense deposits. The thickening and lamellation can be focal or diffuse and alternate with regions of thinning and discontinuity in which the endothelium and epithelium are apposed.[17] Epithelial foot-process fusion is common. A lamellated GBM sometimes occurs in the healing phase of other glomerulonephritides, but lamellation is more widespread and more pronounced in Alport's syndrome.[20] A lamellated or thickened tubular basement membrane (TBM) and Bowman's capsule can also occur in noninherited forms of glomerulonephritis. Basement membranes affected in X-linked Alport's syndrome include those in the glomerulus, cochlea, lens capsule, and retina[21-24] and account for the clinical manifestations seen in this condition. In autosomal recessive and dominant Alport's syndrome, the GBM has been lamellated in all the case reports, but there have been no detailed ultrastructural studies of the renal and extrarenal membranes. In the case of autosomal dominant Alport's syndrome, this occurs partly because the condition is rare or unrecognized and partly because the associated thrombocytopenia often precludes a renal biopsy.

Bull terrier hereditary nephritis represents a model for autosomal dominant Alport's syndrome[25,26] because affected dogs have the typically lamellated GBM and father-to-son disease transmission occurs. Affected animals have hematuria and proteinuria, and all progress to renal failure in adulthood. They are not deaf and do not have the typical hematologic abnormalities, but extrarenal features are variably penetrant in humans and absent from the Samoyed and cocker spaniel models of X-linked and autosomal recessive disease.[27,28] The primary genetic defect in Bull terrier hereditary nephritis is not known, but both the α3(IV) and α5(IV) collagen chains have been demonstrated in affected dog GBM.[26] Bull terrier hereditary nephritis thus provides the opportunity to describe the ultrastructural appearance of the renal basement membranes in a model of autosomal dominant Alport's syndrome and to determine whether the basement membrane abnormality is widespread.

ANIMALS AND METHODS

Dogs

Bull terrier puppies (n = 6) were stillborn (n = 1) or aged 1 day to 12 weeks (n = 5) and were consecutive puppies with one parent with Bull terrier hereditary nephritis and an abnormal GBM ultrastructural appearance in tissue obtained at postmortem examination. An additional four puppies with GBM abnormalities from two litters that had resulted from the mating of two affected Bull terriers were also examined. All puppies examined postnatally had been killed, and specimens were prepared immediately for examination. Proteinuria is common in puppies at some time, and an increased urinary protein-creatinine (UPC) ratio was not used to identify affected animals.

Normal puppies (n = 5) were not Bull terriers and were either cross-breeds or from pedigrees in which there was no known history of renal disease. These animals were age-matched for affected puppies. Bull terriers were not used as controls in this part of the study because hereditary nephritis is widespread in this breed in Australia, and identification of affected animals by definitive genetic testing is not yet available.

Adult affected Bull terriers (n = 18) were identified on the basis of a UPC ratio greater than 0.3, which has previously correlated strongly with histological evidence of Bull terrier hereditary nephritis.[29] Affected dogs were often related to other known affected animals. The corresponding serum creatinine levels varied from normal to very elevated (>1,000 umol/L). Renal biopsies were performed under general anesthetic or when dogs were killed for medical indications.

Normal adult dogs (n = 5) were in general not Bull terriers but were either cross-breeds or from pedigrees in which there was no known history of renal disease. However, the kidneys from one apparently normal adult Bull terrier were available for examination. All normal animals had a UPC ratio less than 0.3.

Histological Examination

Tissues obtained from affected Bull terriers and normal animals were fixed in buffered formalin and processed for light microscopy by standard methods. Three-micron paraffin-embedded sections were stained with hematoxylin and eosin, periodic acid–Schiff, and silver methenamine.

Ultrastructural Examination

Kidney tissue was fixed in 1.5% chilled glutaraldehyde in 0.1 mol/L of phosphate-buffered saline, postfixed in 1% Dalton's osmium tetroxide, and embedded in Epon 812 (TAAB Laboratories, TAAB Lab Equipment, Berkshire, England). Thin sections were cut on Reichert Ultra Cut E microtome (C. Reichert AG, Wien, Austria), supported on 200-mesh copper grids, and stained with saturated uranyl acetate and lead citrate. After carbon coating, the grids were examined in a Philips 301 transmission electron microscope (Phillips Electrical Optics, Eindhoven, The Netherlands).

Other tissues were treated in the same way except that

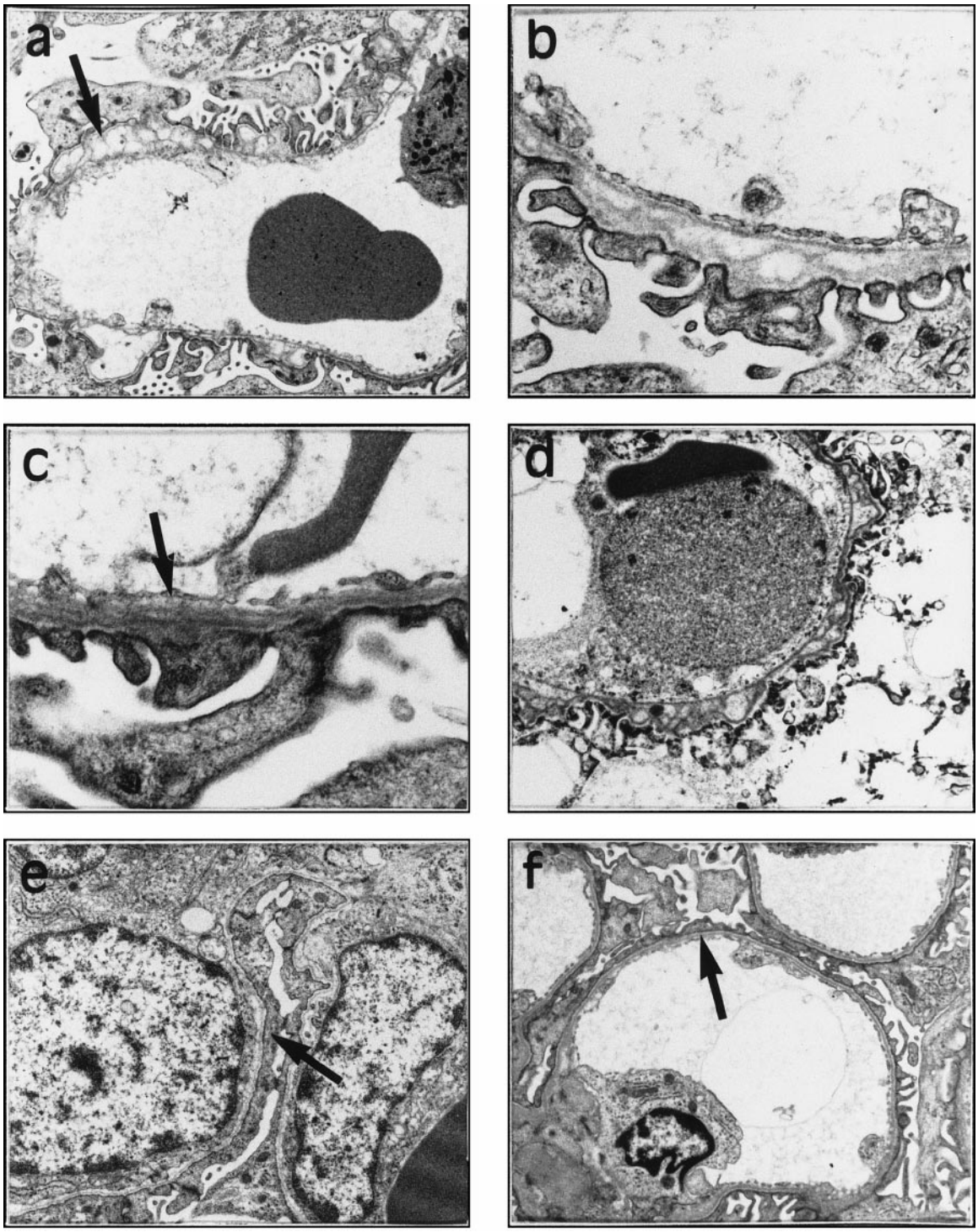

Fig 1. Electron microscopic appearance of kidney basement membranes in affected Bull terrier and normal puppies showing: (a) subepithelial frilling with vacuolation and loops of membrane around the capillary loop (original magnification ×5,100); (b) subepithelial frilling showing membrane on subendothelial and subepithelial surfaces with loops between (original magnification ×14,500); (c) lamellation of the GBM (original magnification ×14,500); (d) subepithelial frilling in a stillborn puppy (original magnification ×5,100); (e) uniform flattening of the foot processes and focal microvillous transformation of the cytoplasm and fetal appearance of podocytes (original magnification ×5,100); and (f) GBM from a normal puppy showing uniform appearance (original magnification ×5,100).

they were postfixed in 1.5% osmium tetroxide and embedded in Spurr's resin. Thin sections were cut on an LKB Ultrotome (LKB Instruments Inc, Gaithersburg, MD), and the grids were examined in a JEOL 1200 EX transmission electron microscope (Japan Electronic Optical Limited, Peabody, WA).

Measurements of Basement Membrane Width

In the dog kidneys examined, electron micrographs were taken of the center and periphery of a capillary loop, overlying Bowman's capsule, and nearby TBM for each of five glomeruli. In addition, basement membranes from the cornea, retina, skin, lung, and muscle were studied.

Basement membrane widths were determined using a magnification and illumination system (Leitz Reprovit R; Leitz Microscope, Weitzmar, Germany) and computer analysis (Videopro 32; Leading Edge Pty Ltd, Adelaide, South Australia). At least 50 measurements of basement membrane width were made at random on electron micrographs of individual tissues from three affected and three normal dogs (except where indicated). A minimum original magnification $\times$15,000 (except for lens capsule, $\times$6,300) was used.

The mean widths for each tissue from affected and normal animals were compared using Student's *t*-test.

Basement Membrane Composition

Affected Bull terrier and normal kidney tissues were obtained immediately after death and fixed in neutral-buffered formalin. Paraffin-embedded sections were treated to remove the paraffin, incubated in 0.2 mol/L of HCl, and heated to 110°C to 127°C for 6 minutes, depending on the requirements of individual antibodies. The sections were then incubated with mouse monoclonal antibodies against the α1(IV) through α6(IV) collagen chains, and the color developed with the LSAB2 kit (Dako, Glostrup, Denmark). The antibodies were made by immunizing mice with synthetic peptides from the carboxyl termini of the human α(IV) noncollagenous domains, and their use has been described previously.[30,31]

RESULTS

Ultrastructural Appearance of Renal Basement Membranes in Affected Bull Terrier Puppies

In affected puppies, the most striking abnormality was subepithelial frilling of the GBM with subepithelial and subendothelial layers (Fig 1) and membrane loops between these that enclosed subepithelial vacuoles and vesicles. These changes were present in all six affected puppies (100%) and were widespread (three of six puppies; 50%), sparse (two of six puppies; 33%), or rare (one of six puppies; 17%). Involvement of different glomeruli was usually patchy. Within individual capillary loops, the changes were not circumferential and were present in both the paramesangium and periphery of capillary loops. The subepithelial frilling and vacuolation were often marked. Identical GBM abnormalities were present in the affected stillborn puppy in which the kidney appearance was clearly embryonic.

Minor degrees of lamellation of the GBM were present in the subepithelial frills, suggesting that the lamellae arose from strands of membrane. Otherwise, lamellation was seen in only one 3-day-old puppy (one of six puppies; 17%), but this was sparse, and subepithelial frilling was also present. Central and subendothelial vacuolation and intramembranous deposits were not shown.

Where there was no subepithelial frilling and vacuolation, the GBM width was normal (Table 1), but elsewhere, it was increased as a result of the subepithelial changes.

Effacement of the epithelial foot processes overlying both abnormal and apparently normal GBM was present in all affected puppies (100%). The mesangial matrix was expanded in two of six dogs (33%). The TBM was uniform and regular, except in one 3-day-old puppy in which there were sparse regions of lamellation (one of six puppies; 17%). The TBM width and the appearance and width of Bowman's capsule were also normal (Table 1).

In four of the five normal puppies (80%), GBM, TBM, and Bowman's capsule had no abnormalities, but in the fifth puppy (one of five puppies; 20%), there were rare and mild examples of subepithelial irregularity.

One puppy from each of the two litters in which both parents were affected was much smaller than the other, with small limbs and a

Table 1. Basement Membrane Widths in Affected Bull Terrier and Normal Puppies

	Width (nm)	
Basement Membrane	Affected Puppies (n = 4)	Normal Puppies (n = 3)
GBM (random)	171 $\pm$ 41*	122 $\pm$ 12
GBM (no subepithelial frilling or vacuolation)	135 $\pm$ 8.5	122 $\pm$ 12
TBM	190 $\pm$ 48	129 $\pm$ 61
Bowman's capsule	730 $\pm$ 313	797 $\pm$ 223

NOTE. The width of the basement membrane itself was measured. Values expressed as mean $\pm$ SD.

* $P < 0.05$ compared with normal puppies; otherwise not significant.

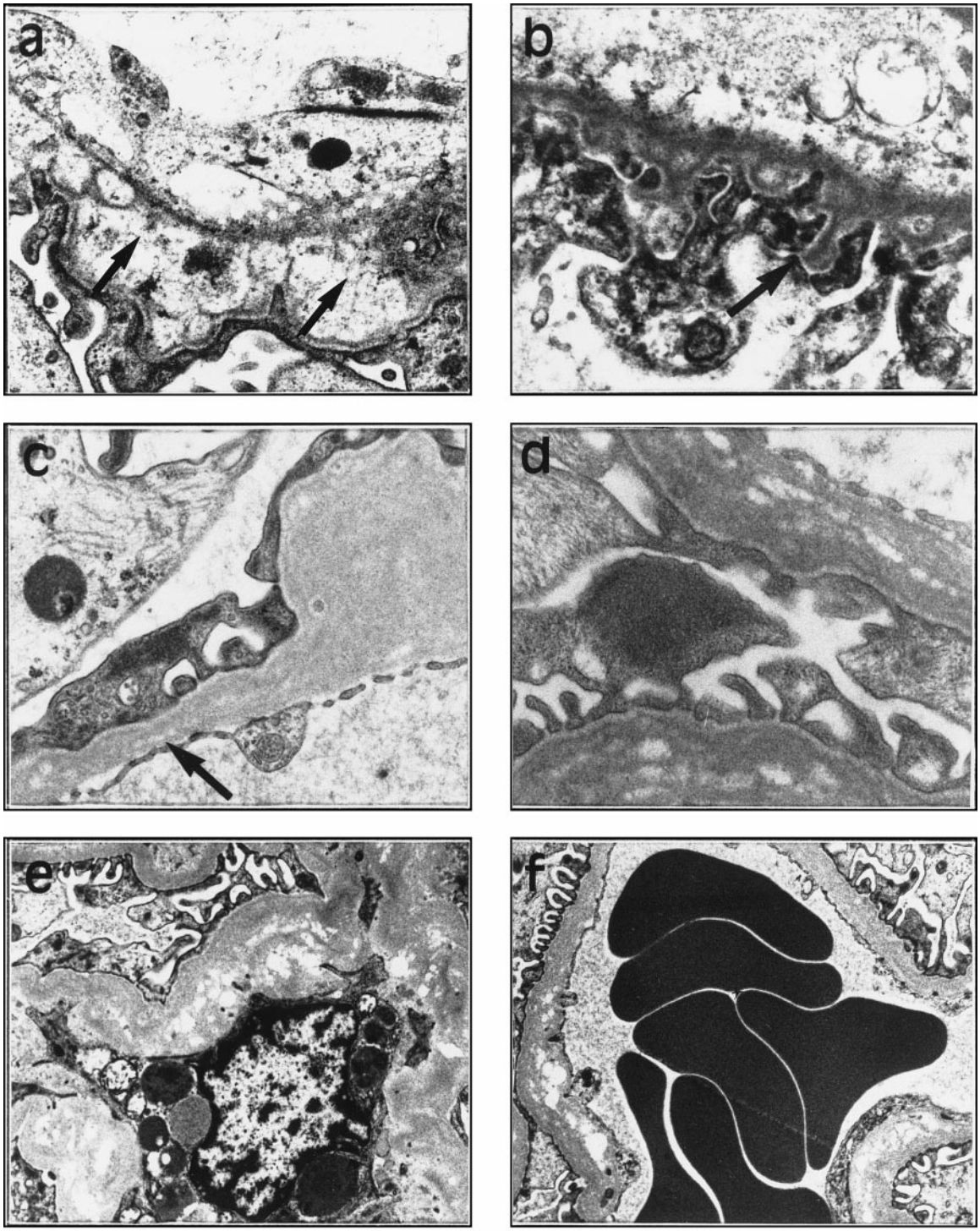

Fig 2. Electron microscopic appearance of kidney basement membranes in affected adult Bull terriers showing: (a) irregular lucency on the epithelial aspect of the GBM separated by a peripheral band of membrane from adjacent epithelial cells (original magnification ×14,500); (b) subepithelial lucency separated from overlying epithelial cells by membrane that is also found between epithelial cells (arrow) (original magnification ×14,500); (c) in this older dog, the lucent zones are both subepithelial and central (arrow), and there is a dense nodule protruding from the epithelial aspect of the GBM; this appearance may represent a late healing phase of the subepithelial lesion (original magnification ×14,500); (d) the GBM is widened by variably laminar and vacuolar lucencies, and there is no subepithelial irregularity (original magnification ×14,500); (e) there are numerous predominantly microvesicular lucencies in the GBM, especially prominent adjacent to the mesangium (original magnification ×5,100); (f) the lucencies extend from the paramesangial areas into the peripheral GBM, with some on the subendothelial aspect (original magnification ×5,100).

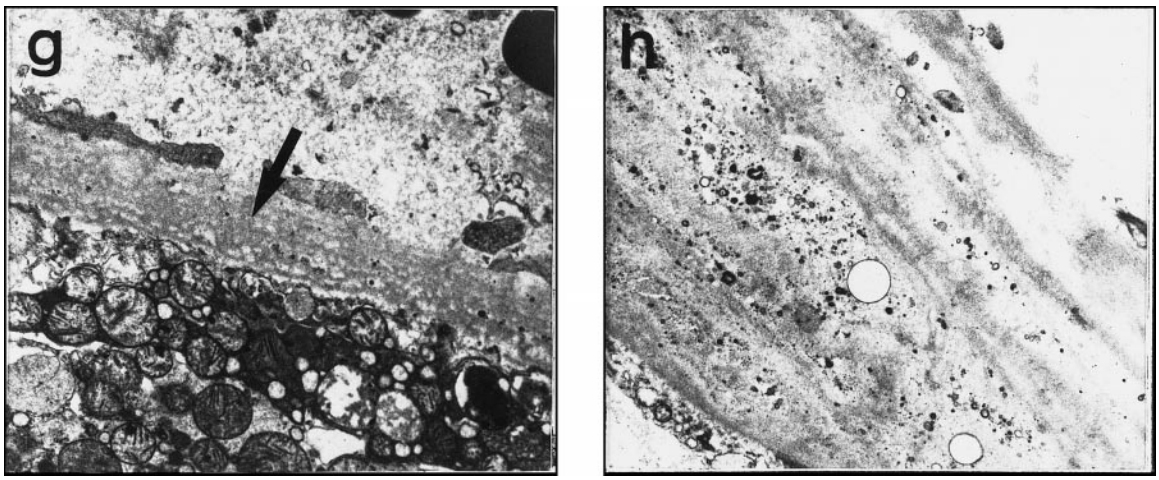

Fig 2. (Cont'd). (g) thickened lamellated TBM (arrow) (original magnification ×14,500); and (h) thickened Bowman's capsule with mineralization (original magnification ×14,500).

form of spina bifida but without macroscopic abnormalities in other organs at postmortem examination. In one litter, both puppies had extensive and marked subepithelial changes in the GBM that were similar to the more severe lesions previously described. In addition, there was epithelial foot-process effacement and mild mesangial expansion, but the TBM and Bowman's capsule were normal. In the other litter, the puppy with the skeletal abnormalities had moderate subepithelial changes in the GBM, and its littermate had less marked features. Again, there was foot-process effacement, but the TBM and Bowman's capsule were normal in both animals. Thus, the extent and severity of the GBM ultrastructural changes did not differ between puppies with skeletal abnormalities that were possibly homozygous for the underlying genetic mutation and their littermates or the affected offspring of a single affected parent.

Ultrastructural Appearance of Renal Basement Membranes in Affected Adult Bull Terriers

In affected adult dogs, subepithelial frilling (16 of 18 dogs; 89%), vacuolation (15 of 18 dogs; 83%), and lamellation (11 of 18 dogs; 61%) were present in the GBM. In the 2 dogs without subepithelial frilling (11%), vacuolation and lamellation were present. All examined glomeruli from each dog were abnormal, but involvement was patchy (Fig 2).

The subepithelial frilling was more extensive but less prominent than in the puppies. Again, the frilling in individual glomeruli was not circumferential and affected both the paramesangium and periphery of capillary loops. In the younger adult animals, there was often a complex spicular arrangement of newly formed GBM lying between adjacent foot processes similar to that seen in membranous nephropathy but without the characteristic deposits. This appearance suggested active formation of new GBM by epithelial cells adjacent to the lucent zones. Some animals had scattered nodular protrusions of variably dense GBM, which may have represented a later stage of the subepithelial lesion.

The vacuoles were smaller and more regular than in the puppies. Again, the lesions were not circumferential and affected both the paramesangium and peripheral capillary loops. Lucencies were present in 15 of 18 dogs (83%) and were centrally located within the GBM (15 of 18 dogs; 83%) or in a central and subendothelial location (3 of 18 dogs; 17%). The lucencies were vacuolar, vesicular, microvesicular, or lamellar in nature. Some dogs had extensive central lucency of the GBM with a multivesicular bubbly pattern, especially around the mesangium. There were occasional electron-dense deposits within the vacuoles.

A lamellated GBM was more common in affected adult dogs (11 of 18 dogs; 61%) than in puppies (1 of 6 puppies; 17%), and the lamellation was often extensive. Sometimes the lamel-

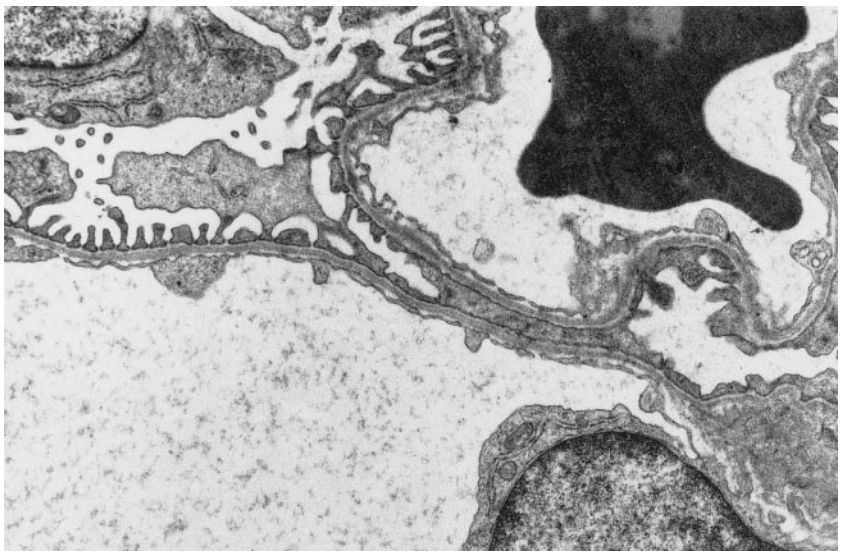

Fig 3. Electron microscopic appearance of GBM in normal adult Bull terriers showing a uniform GBM (original magnification ×12,600).

lae comprised layers of small vesicles between sheets of membrane.

The extent and nature of the GBM lesions were not different in male and female adult dogs. In the males, subepithelial lesions were present in 9 of 10 animals (90%), with central lucencies in 5 of 10 animals (50%), central and subendothelial lucencies in 3 of 10 animals (30%), and lamellation in 5 of 10 animals (50%) compared with females, who had subepithelial lesions in 7 of 8 animals (88%), central lucencies in 6 of 8 animals (75%), no central and subendothelial lucencies (0 of 8 animals; 0%), and lamellation in 6 of 8 animals (75%). The abnormalities were present in all glomerular loops examined in the adult dogs (17 of 17 dogs; 100%) compared with puppies (3 of 6 puppies; 50%), and subepithelial frilling was less prominent in adult animals but still frequently seen (16 of 18 animals; 89%) compared with puppies (6 of 6 puppies; 100%), whereas central and subendothelial lucencies were present only in adult dogs, and lamellation was more common (11 of 18 animals; 61%) compared with puppies (1 of 6 puppies; 17%).

The GBM ultrastructural changes were compared among affected dogs within pedigrees. Two half-sibs had subepithelial GBM changes, central lucencies, and lamellation, and in a second pedigree, the mother had subepithelial changes, central lucencies, and lamellation, whereas the daughter had subepithelial changes only.

Again in affected dogs, the presence of subepithelial frilling, vacuolation, and lamellation meant that the width of the GBM in affected dogs was significantly increased compared with that in normal animals (Fig 3 and Table 2).

Foot-process effacement overlying both abnormal and normal GBM and mesangial matrix expansion was common. The TBM was lamellated in 3 of the 17 adult dog kidneys (18%) in which it was examined. Bowman's capsule was

Table 2. Basement Membrane Widths in Different Tissues in Affected Adult Bull Terriers and Normal Dogs

	Width (nm)	
Basement Membrane	Affected Adult Bull Terriers (n = 3)	Normal Dogs (n = 3)
GBM	554 ± 99*	305 ± 39
TBM	988 ± 190†	467 ± 127
Bowman's capsule	2,312 ± 357†	1,088 ± 409
Cornea	114 ± 17	102 ± 4
Retina	107 ± 41	90 ± 6
Skin	89 ± 17	68 ± 18
Lung	156 ± 40	115 ± 42
Muscle	85 ± 38	61 ± 18

NOTE. Results were calculated from the means of measurements made on three affected and three normal dogs. Values expressed as mean ± SD.

* $P < 0.02$ compared with normal dogs; otherwise not significant.

† $P < 0.05$ compared with normal dogs; otherwise not significant.

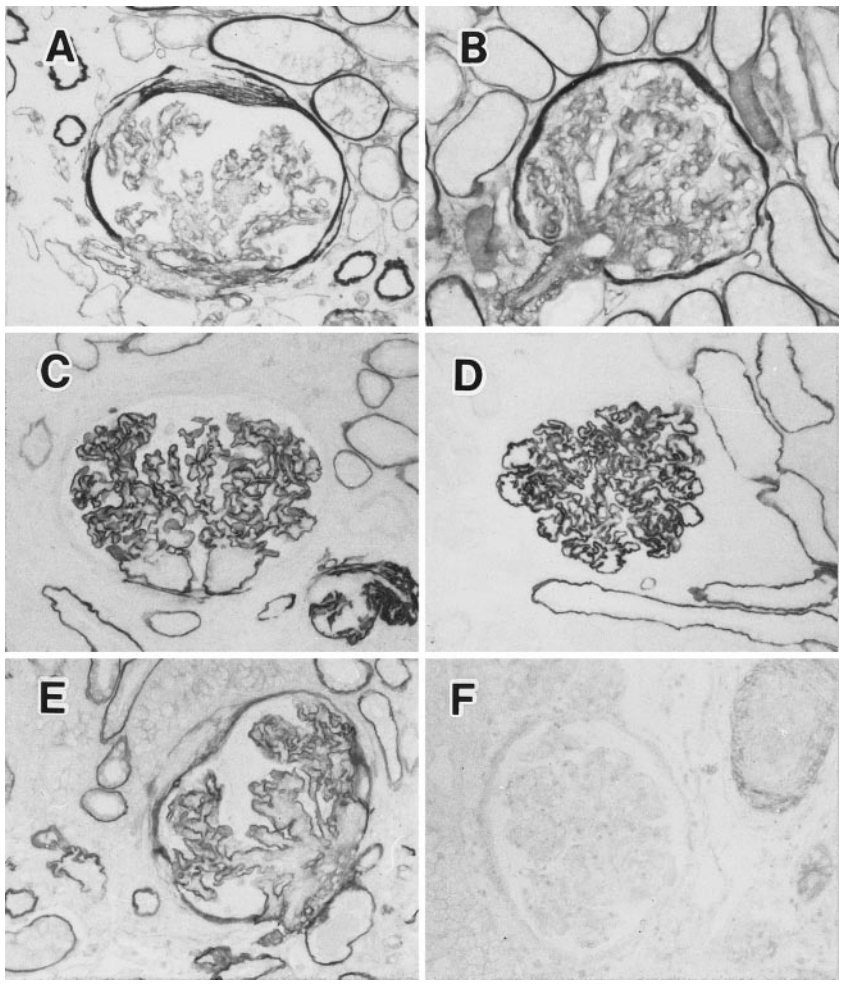

Fig 4. (A-F) Immunofluorescent examination of affected dog kidney stained with antibodies against α1(IV) through α6(IV) collagen chains shows that all these collagen chains are present.

split in 16 of the 18 kidneys examined (89%). The widths of the TBM and Bowman's capsule in adult dogs were significantly increased compared with normal animals (Table 2), and the GBM, TBM, and Bowman's capsule were commonly mineralized in adult affected dogs.

Four of the five normal dogs (80%) had no GBM abnormalities, but one had minor rare subepithelial irregularity (1 of 5 animals; 20%), and another had minor TBM lamellation (1 of 5 animals; 20%). Bowman's capsule appeared split in some places in all normal dogs.

The GBM from the normal Bull terrier was uniform in appearance (Fig 3).

Basement Membrane Composition

Each of the α1(IV) through α6(IV) collagen chains was shown in renal basement membranes from affected dogs (Fig 4). The α1(IV) and α2(IV) chains were present in all basement membranes; the α3(IV) through α5(IV) chains in the GBM, Bowman's capsule, and distal TBM; and a tiny amount of the α6(IV) chain was shown in Bowman's capsule and also appeared to be present in the vascular basement membrane. This distribution was identical to that seen in normal dogs.

Ultrastructural Appearance of Extrarenal Basement Membranes in Adult Affected Dogs

The light microscopic appearance of the cornea, lens, retina, skin, lung, and muscle in affected dogs was normal (Fig 5) and did not differ from the appearance in normal animals. The ultrastructural appearance of the basement membranes in these tissues was generally normal, without lamellation, thickening, or thinning. There were rare focal regions of lamellation in

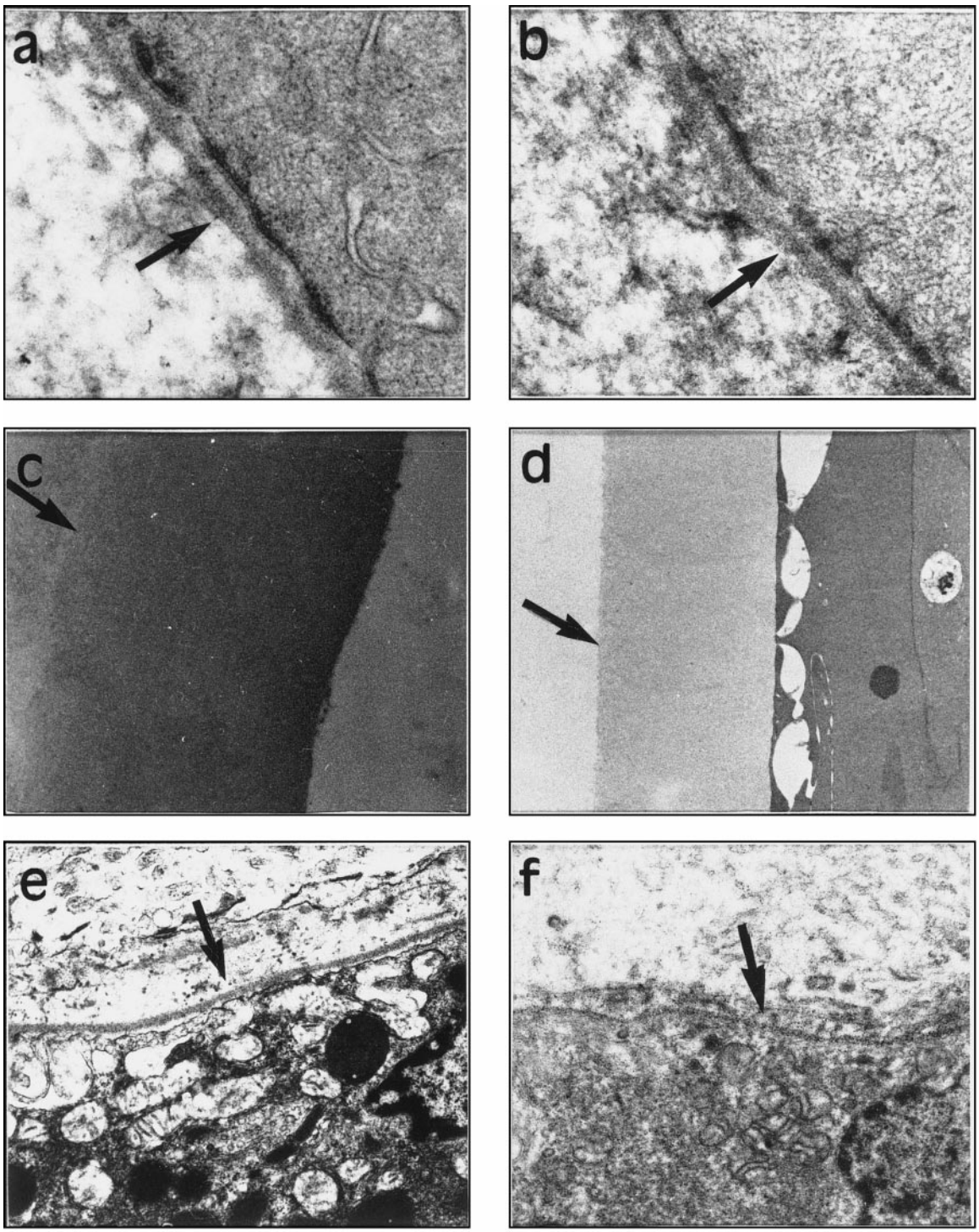

Fig 5. Electron microscopic appearance of the basement membranes (arrows) in (a) cornea (normal; original magnification ×63,000); (b) cornea (affected; original magnification ×63,000); (c) lens capsule (normal; original magnification ×6,300); (d) lens capsule (affected; original magnification ×6,300); (e) retina (normal; original magnification ×15,120); (f) retina (affected; original magnification ×15,120).

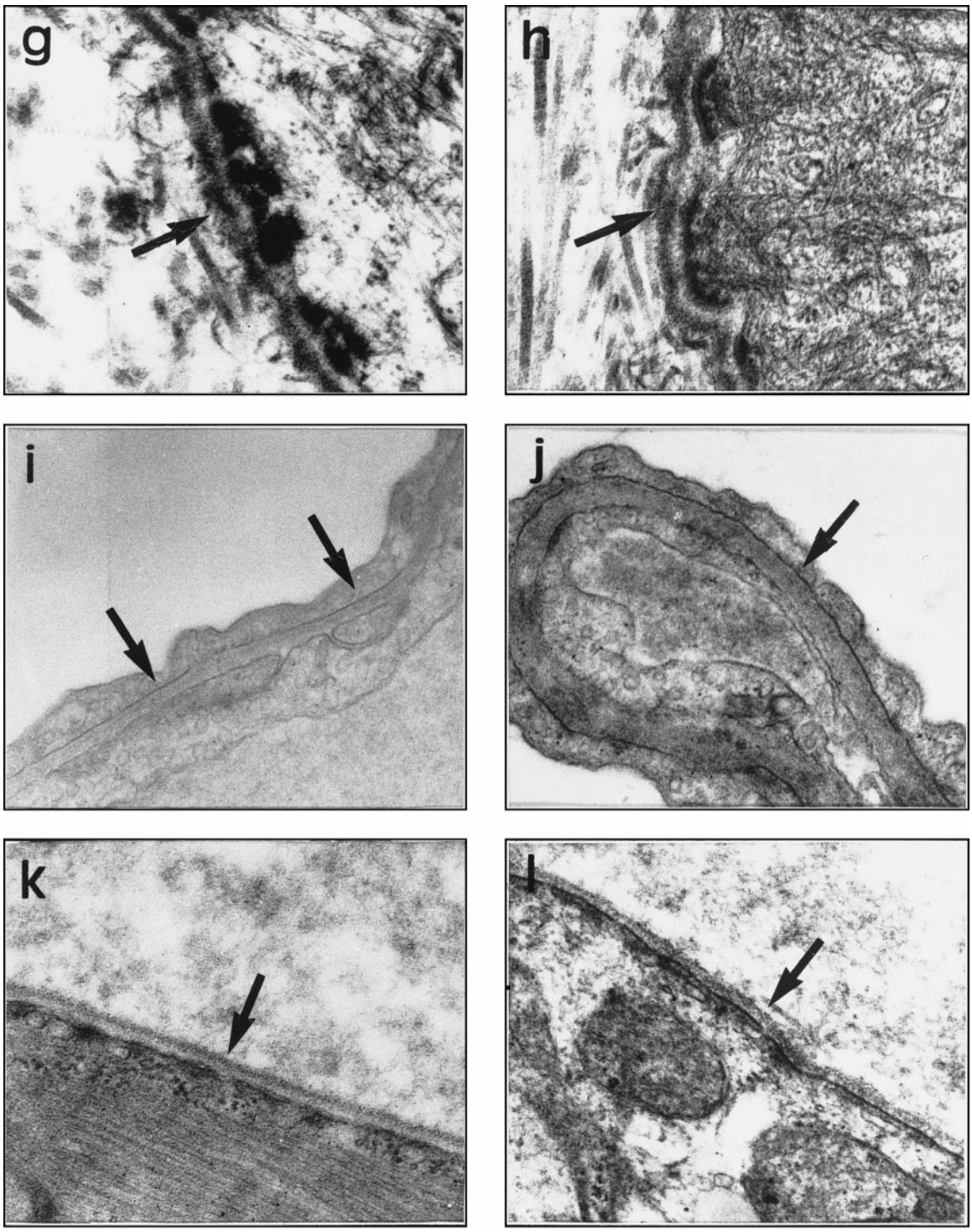

Fig 5 (Cont'd). (g) kin (normal; original magnification ×63,000); (h) skin (affected; original magnification ×63,000); (i) lung (normal; original magnification ×42,000); (j) lung (affected; original magnification ×42,000); (k) muscle (normal; original magnification ×63,000); and (l) muscle (affected; original magnification ×63,000).

the alveolar basement membranes in two affected but no normal dogs. These lesions were not considered to reflect Alport's syndrome because they were not generalized.

The widths of the basement membranes from the cornea, retina, skin, lung, and muscle did not differ significantly from those in normal animals (Table 2).

DISCUSSION

This study has shown that the GBM in Bull terriers with autosomal dominant hereditary nephritis is abnormal from birth, with subepithelial frilling and bubbly vacuolation but without thinning. The only descriptions of GBM in children with Alport's syndrome are in the X-linked form, in which the basement membrane is thinned,[32] but thinning does not occur in the Samoyed or cocker spaniel models of X-linked and autosomal recessive disease. The GBM in affected adult Bull terriers is lamellated, but the subepithelial expansion and vacuolation, although extensive, is less prominent than in puppies. The occasional case reports of adults with autosomal dominant Alport's syndrome indicate that the GBM is lamellated, with minor subepithelial frilling identical to that seen in X-linked and autosomal recessive disease[9,10] and in the corresponding canine models.[27,28] The differences between the ultrastructural abnormalities in affected puppies and, to a lesser extent, adult Bull terriers and human disease may result from differences in the composition of dog and human GBM, the nature of the protein abnormalities in these diseases, or the expression of affected proteins at different stages of development of the kidney.

Affected Bull terriers are descendants of a common forebear and are assumed to have the same genetic mutation; however, the ultrastructural appearance of the GBM varied between different dogs and between parents and offspring. Thus, factors other than the genetic mutation must contribute to the nature and extent of the GBM ultrastructural lesions. We found no difference in the GBM lesions between male and female Bull terriers, but the abnormalities were different and more extensive in adult animals compared with puppies. As with thinning in human X-linked disease, the subepithelial frilling and vacuolation seen in Bull terrier puppies may reflect abnormal membrane synthesis,[32] whereas the lamellation in adult dogs may represent an attempt at membrane repair. We have not examined dogs for worsening of the GBM lesion with increasing age, but in X-linked Alport's syndrome and the Samoyed model, GBM lamellation increases with deteriorating renal function,[18] becoming more extensive and more prominent.[27] Other factors that may contribute to variability of the ultrastructural appearance of the GBM in different Bull terriers include environmental and local intrarenal stresses.

In X-linked Alport's syndrome, the extent of the GBM abnormalities partly depends on whether the individual is a male with a single copy of the abnormal gene and no normal gene, or a heterozygous female. The effect of a double dose of the mutation has not been described in autosomal dominant Alport's syndrome but occurs with autosomal recessive inheritance, which may affect the same gene. Each litter of two affected Bull terrier parents was small, implying that the parents were less fertile or that homozygous offspring were less viable than normal. One puppy from each litter was much smaller than the other, with marked skeletal abnormalities. Skeletal defects are not uncommon in dogs but have not been described in a form of Alport's syndrome, and it is not clear that the abnormal protein in autosomal dominant disease is present in bone. The GBM of all four offspring in these litters had an abnormal ultrastructural appearance, but there was no difference in the nature and extent of the abnormalities between littermates nor differences from those seen in the affected offspring of a single affected parent. These observations suggest that none of the puppies examined was homozygous for the mutant gene or that there is no difference between the GBM abnormalities in heterozygous and homozygous animals.

As well as the abnormalities seen in the GBM, the TBM was lamellated in at least one Bull terrier puppy. A lamellated TBM has been described in X-linked Alport's syndrome[19] but can also be normal. The thickened TBM noted in adult Bull terriers is a common finding with many forms of glomerular damage.

In Bull terrier puppies with autosomal dominant Alport's syndrome, Bowman's capsule was

not lamellated or vacuolated, but in adult dogs, it was thickened, probably nonspecifically. In affected adult dogs, the GBM, TBM, and Bowman's capsule were calcified, which again probably represents a nonspecific finding related to renal impairment. The vascular membranes were not examined.

An additional ultrastructural abnormality noted in Bull terrier kidneys was epithelial foot-process fusion, which was present in puppies only a few weeks old, as well as in older dogs. This was shown overlying both normal and abnormal GBM and has been well described in X-linked disease.[19]

The immunohistochemical studies described here indicated that all type IV collagen chains were present in affected Bull terrier GBM. These findings thus confirm a third pattern of type IV collagen staining in Alport's syndrome. In males with X-linked Alport's syndrome, the α3(IV) through α5(IV) collagen chains are usually but not always absent from the GBM[6,30]; in autosomal recessive disease, the α3(IV) through α5(IV) chains are often absent from the GBM, but the α5(IV) chain is present in Bowman's capsule[8]; and in autosomal dominant disease, the α3(IV) through α5(IV) chains are present in affected GBM.[14]

Thus, there are differences between the clinical features and GBM appearance in humans with autosomal dominant Alport's syndrome and Bull terriers with hereditary nephritis. These distinctions may result from different proteins being affected or from differences in protein expression between the species. The demonstration of all type IV collagen chains in affected Bull terrier GBM does not preclude a mutation in one of the corresponding genes, but linkage studies have suggested that a novel gene is involved.[16]

In the Bull terrier model of hereditary nephritis, the kidney was the only tissue with basement membranes containing the Goodpasture antigen[26] and the α3(IV) through α5(IV) collagen chains that had an abnormal light microscopic appearance.

In affected Bull terriers, the ultrastructural appearance of the cornea, lens capsule, retina, skin, lung, and muscle was normal, without lamellation or thickening. These tissues were examined either because they corresponded to clinical manifestations in X-linked disease or because their basement membranes had a similar composition to the membranes affected in this condition. The thickness of the lens capsule was not measured because it depends precisely on the site of sampling, but thinning has been observed in human X-linked disease.[33] The retinal basement membrane is normal in affected male Samoyeds.[34] The corneal membrane has not been studied in human X-linked disease or Samoyeds, and the associated clinical manifestation of posterior polymorphous corneal dystrophy is rare.[35] The other common associations of autosomal dominant Alport's syndrome are deafness and platelet defects, but Bull terriers are not deaf and have morphologically and functionally normal platelets. The basement membranes of the stria vascularis in the dog ear were not examined because of difficulties obtaining and fixing the tissue but they are abnormal in humans and Samoyeds with X-linked disease.[34] Ultrastructural studies of platelets in humans with autosomal dominant disease have been inconclusive.[36]

Affected Bull terriers had focally lamellated and thickened lung basement membranes, but these changes were sparse and hence were not considered typical of Alport's syndrome. The lung basement membranes have not been examined in patients with X-linked or autosomal recessive disease but are normal in male Samoyeds.[34]

We were unable to show ultrastructural changes in the skin, and the results of studies of X-linked disease have been inconclusive,[36] or normal in male Samoyeds.

Thus, the basement membrane abnormalities in autosomal dominant Alport's syndrome affect the GBM but not the basement membranes of the lens, cornea, retina, skin, lung, or muscle. Membrane abnormalities may occur because the abnormal protein is present exclusively or more abundantly, or is structurally more important in the kidney, or because of the effect of local intrarenal stresses. Observations made in this study provide the impetus to examine whether the ultrastructural membrane changes in human autosomal dominant Alport's syndrome are present before birth, whether these abnormalities vary between different individuals, and whether the extrarenal membranes are normal when there are no corresponding clinical features.

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