A novel model of autosomal dominant Alport syndrome in Dalmatian dogs

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Abstract

Background. Autosomal dominant Alport syndrome is a rare inherited disease characterized clinically by haematuria, renal failure and deafness, and ultrastructurally by a lamellated glomerular basement membrane (GBM). It is usually caused by mutations in the COL4A3 or COL4A4 genes which code for the α3 and α4 chains of type IV collagen. We describe here a novel spontaneous model of autosomal dominant Alport syndrome in Dalmatian dogs.

Methods. Affected dogs were identified by a urinary protein:creatinine ³0.3. A total of 10 affected adult Dalmatians and eight unaffected age- and sex-matched dogs from breeds other than Dalmatians were examined. In addition, kidneys from five Dalmatian fetuses from affected mothers were examined histologically and ultrastructurally.

Results. All affected dogs were purebred Dalmatians and had a common progenitor. Successive generations were affected, and males and females were affected equally often and equally severely, consistent with autosomal dominant inheritance. The median age at onset of renal failure was 18 months (range 8 months to 7 years). Affected dogs were not clinically deaf, and did not have the ocular abnormalities seen in human X-linked or autosomal recessive Alport syndrome. In addition, they did not have the leucocyte inclusions, low platelet counts or large platelets seen in autosomal dominant hereditary nephritis due to MYH9 mutations. The renal histology and ultrastructural appearance of the GBM appeared to be normal in utero. However, affected adult kidneys demonstrated segmental glomerular hyalinosis and sclerosis with tubulo-interstitial inflammation and fibrosis, and on ultrastructural examination the GBM was lamellated with subepithelial frilling, vacuolation and occasional intramembranous deposits. All α1(IV)–α5(IV) type IV collagen chains were present in the affected GBM and Bowman’s capsule.

Conclusions. Autosomal dominant Alport syndrome in Dalmatians resembles the disease in Bull terriers but has arisen independently. These models will enable us to determine how genetic mutations affect the corresponding proteins and overall membrane structure in autosomal dominant Alport syndrome.

Keywords: autosomal dominant Alport syndrome; Dalmatian dogs; glomerular basement membrane; hereditary nephritis

Introduction

Most individuals with Alport syndrome have X-linked or autosomal recessive disease which is characterized clinically by haematuria, progressive renal failure, hearing loss, lenticonus and dot-and-fleck retinopathy [1,2]. The glomerular basement membrane (GBM) is typically lamellated with vacuolation and subepithelial frilling [3]. In X-linked Alport syndrome, mutations affect the COL4A5 gene [4] and result in the loss of the α5(IV) and α4(IV) collagen chains from the affected GBM and Bowman’s capsule. Mutations in COL4A3 and COL4A4 genes [6] which again usually cause the loss of the α3(IV)–α5(IV) collagen chains from affected glomerular membranes [7].

Autosomal dominant Alport syndrome is rare, and the clinical phenotype differs from that seen with X-linked and autosomal recessive inheritance. Haematuria is associated with both normal renal function and progressive renal impairment, hearing loss is common, but ocular abnormalities do not occur [8,9]. Again, the GBM is lamellated but the α3(IV)–α5(IV) collagen chains are present in the affected GBM [10]. Mutations affect the COL4A3 or COL4A4 genes [8,9] which are
also abnormal in autosomal recessive inheritance. Autosomal dominant Alport syndrome has to be distinguished from autosomal dominant hereditary nephritis with progressive renal failure, deafness, and leucocyte inclusions, low platelet counts and large platelets [11,12]. This is caused by mutations in the MYH9 gene which encodes a non-muscle myosin heavy chain [13,14].

The diagnosis of all forms of Alport syndrome depends on the presence of the typical clinical features together with a family history of the disease, or on the ultrastructural demonstration of a lamellated GBM. It is always caused by a mutation in one of the type IV collagen genes. Autosomal dominant inheritance is characterized by disease in successive generations, and males and females being affected equally often and equally severely. It is the only form of inheritance in which disease is transmitted from father to son.

Canine models of X-linked, autosomal recessive and dominant Alport syndrome in Samoyed and Navasota dogs, Cocker spaniels and Bull terriers, respectively [15–19], have contributed enormously to our understanding of the pathogenesis and pathology of Alport syndrome. We describe here a novel canine model of autosomal dominant Alport syndrome in Dalmatians and compare its clinical, histological and ultrastructural features with those seen in the Bull terrier model and the human disease.

**Animals and methods**

**Dogs**

Affected adult Dalmatians \((n=10)\) were identified by a urinary protein creatinine ratio (UPC) \(>0.3\), which has previously corresponded with histological evidence of renal disease in Bull terrier hereditary nephritis [19,20]. Some animals had, in addition, laboratory evidence of renal failure or the typical light microscopic abnormalities. Normal adult dogs \((n=8)\) were either cross-breeds or from pedigrees where there was no known history of renal disease, and were approximately the same age and size as adult Dalmatians. Normal animals had a UPC \(<0.3\) on two occasions at least 1 month apart, no laboratory evidence of renal failure, and renal biopsies with a normal light microscopic and ultrastructural appearance.

Dalmatian fetuses \((n=5)\) were from litters with an affected mother and were aged \(\sim 35\) days (canine gestation is 61–63 days). Normal fetuses \((n=5)\) were also at about 35 days of gestation, and were from dog breeds where there was no known history of renal disease. Fetuses were obtained either at the time of routine hysterectomy or after euthanasia for medical reasons.

**Clinical features**

Random voided urine specimens were tested for haematuria and UPC. Haematuria was present if the urine was positive for blood on testing with Multistix (Ames Co., Miles Laboratories, Elkhart, IN, USA) or if any red cells were observed in a field at \(\times 600\) magnification by light microscopy. UPC was estimated using a Cobas Mira autoanalyser (Hoffman–LaRoche, Basel, Switzerland) and the results confirmed on a second specimen taken at least 1 month later.

Hearing was tested clinically at 4 weeks by a veterinarian. Eyes were dilated with topical 1.0% tropicamide and examined by direct ophthalmoscopy by an experienced observer for the ‘oil droplet’ sign of anterior lenticonus and the dot-and-fleck retinopathy seen in human X-linked and autosomal recessive Alport syndrome. Blood films were made within 3 h of collection, stained with the May Grunwald Giemsa stain, and examined for neutrophil inclusions and platelet number and size.

**Histological examination**

Renal tissue obtained from biopsies, collected under general anaesthesia or after euthanasia for medical indications, was fixed immediately in neutral-buffered formalin and processed for light microscopy by standard methods. Three micron paraffin-embedded sections were stained with haematoxylin and eosin, periodic acid-Schiff and silver methenamine.

**Ultrastructural examination**

Renal tissue was fixed in 1.5% chilled glutaraldehyde in 0.1 M phosphate-buffered saline, post-fixed in 1% Dalton’s osmium tetroxide and embedded in Epon 812 (TAAB Laboratories, TAAB Lab Equipment, Berkshire, UK). Thin sections were cut on a Reichert Ultra Cut E microtome, 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.

**Basement membrane composition**

Fixed, embedded blocks of kidney tissue were incubated in 0.2 M HCl at 110–127°C for 6 min depending on the requirements of individual antibodies. Sections were then incubated with rat monoclonal antibodies against the \(\alpha(IV)\)-\(\alpha(IV)\)-non-collagenous \(\alpha(IV)\) collagen chains and the colour developed with the LSAB2 kit (DAKO, Glostrup, Denmark). These antibodies had been prepared by immunizing rats with synthetic peptides from the C-termini of the human \(\alpha(IV)\)-collagenous domains. Their use has been described previously [21].

This project had the approval of the Animal Ethics’ Committee of Murdoch University.

**Results**

**Clinical features**

Ten affected adult dogs comprising five males and five females were examined over a 10 year period. All affected dogs were purebred Dalmatians, and their pedigrees demonstrated a common progenitor and affected dogs in successive generations.

Haematuria was present in four of the five affected dogs (80%) tested, and none of the normals. Four males and four females had renal failure. Their median age at onset of renal failure was 18 months (range 8 months to 7 years). None of the affected dogs was clinically deaf at 4 weeks of age \((n=10)\) or subsequently demonstrated any features suggesting deafness to their
owner-breeders, and none had the ocular abnormalities seen in human X-linked or autosomal recessive Alport syndrome ($n=6$). In addition, none had the leucocyte inclusions, thrombocytopenia or large platelets present in autosomal dominant hereditary nephritis with haematological abnormalities due to MYH9 mutations ($n=6$).

**Histological and ultrastructural appearance**

**Adult kidneys.** The light microscopic appearance was nearly normal in biopsies from dogs with early disease ($n=3$). Later, at post-mortem ($n=4$), the kidneys were macroscopically small with moderate pallor and firmness. Histologically there was segmental hyalinosis and glomerular sclerosis, tuft adhesions without an increase in tuft cellularity, mild through to marked tubulo-interstitial inflammation and fibrosis, and gross capillary wall thickening.

Ultrastructural examination of the GBM from biopsies from affected dogs early in the disease and later at post-mortem showed lamellation, subepithelial frilling and vacuolation (Figure 1). These abnormalities all increased the width of the affected GBM compared with normal. The frilling affected both the parameasangium and the peripheral capillary loops in individual glomeruli. Vacuolation and occasional intramembranous deposits were present. Foot process effacement overlying the abnormal GBM, and mesangial matrix expansion were noted. The ultrastructural abnormalities were, however, less prominent than in affected Bull terriers since some glomerular loops from each dog were only mildly abnormal, and GBM changes often affected only part of the circumference of the glomerular loop. The tubular basement membranes and Bowman’s capsule were of normal thickness and appearance in the affected kidneys.

Fig. 1. Ultrastructural appearance of glomerular basement membrane from affected Dalmatians with autosomal dominant Alport syndrome: (A) normal membrane in 35-day-old embryo ($\times 5700$); (B) lamellated membrane with fusion of overlying foot processes in adult dog ($\times 1850$); (C) irregular thickened lamellated membrane with subepithelial frilling and fusion of foot processes in adult dog ($\times 1850$); and (D) membrane showing irregular basket weave appearance in adult dog ($\times 5700$).
The normal dogs had no GBM abnormalities, but one had a minor rare subepithelial irregularity and another had a minor TBM lamellation. Bowman’s capsule appeared split in some places in all dogs.

Fetal kidneys. The light microscopic appearance was normal in all five fetuses from litters where one parent was affected, and ultrastructural examination showed that the GBM had none of the subepithelial frilling, lamellation or vacuolation seen in affected neonatal Bull terriers. The absence of ultrastructural GBM changes in these embryos did not, however, preclude in utero abnormalities in this model since it was not possible to confirm that any fetus was affected. The histological and ultrastructural appearances of the normal fetal kidneys (n = 5) showed no significant abnormalities.

Basement membrane composition

Each of the x1(IV)–x6(IV) collagen chains was demonstrated in the renal basement membranes from affected dogs. The x1(IV)–x2(IV) chains were present in all the basement membranes, the x3(IV)–x5(IV) chains in the GBM, Bowman’s capsule and the distal TBM, and a tiny amount of the x6(IV) chain was demonstrated in Bowman’s capsule. This distribution was identical to that seen in Bull terriers with hereditary nephritis and in normal dogs.

Discussion

In affected Dalmatians, the diagnosis of Alport syndrome and its autosomal dominant nature were confirmed by the demonstration of GBM lamellation, and by the presence of disease affecting males and females in successive generations equally often and equally severely. Autosomal dominant Alport syndrome in Dalmatians resembles the human disease with affected dogs developing haematuria, progressive renal failure, glomerular sclerosis and tubulo-interstitial fibrosis, and having a lamellated, vacuolated GBM that contains all type IV collagen chains. While affected dogs do not have the hearing loss found in human disease, deafness is also absent from affected dogs in both the Dalmatian and Bull terrier models of autosomal dominant Alport syndrome. The only distinguishing features are the possible absence of ultrastructural changes in utero in Dalmatians and their less prominent GBM abnormalities. All affected Dalmatians and Bull terriers have arisen from two independent progenitors in each breed within the past 50 years, and are likely to have two different mutations. The mutation in Bull terriers is also likely to affect the COL4A3 or COL4A4 genes and the minor differences in phenotype could be due to the small number of Dalmatians examined, differences in the underlying mutations or to environmental effects.

The abundance of canine models of Alport syndrome may reflect an increased mutation rate in the causative genes, the care with which inherited disease is monitored and investigated in pedigree dogs, or the sophistication of diagnostic techniques available to veterinary medicine. The Bull terrier model of autosomal dominant Alport syndrome in particular has contributed enormously to our understanding of a condition that is rare or infrequently recognized. The Bull terrier model has suggested that the abnormalities in autosomal dominant Alport syndrome begin in utero, that impaired renal function is principally due to tubulo-interstitial disease, and that extrarenal basement membranes are not affected [18–21,23].

The Dalmatian and Bull terrier models of autosomal dominant Alport syndrome can be used to explore the relationship between genetic mutations, the corresponding biochemical defects, and the clinical and ultrastructural phenotypes.

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References


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