

## **Familial Nephropathy (FN) in [English] Cocker Spaniel Dogs**

George E. Lees, DVM, MS, DACVIM; Texas A&M University, USA

Email: [glees@cvm.tamu.edu](mailto:glees@cvm.tamu.edu) Telephone: 979-845-2351

### **Introduction**

An inherited renal disease that causes juvenile-onset renal failure has been recognized in Cocker Spaniels (known in North America as English Cocker Spaniels) worldwide for more than 50 years. During the last decade, research has demonstrated that the disease is caused by certain molecular abnormalities of the collagen content of the walls of the glomerular capillaries through which blood filtration occurs in the kidneys. This renal disease is analogous to a condition called Alport syndrome that occurs in people.

Glomerular capillary walls consist of several layers; inner and outer layers of cells, and a middle layer of extracellular matrix material called the glomerular basement membrane (GBM). The GBM contains a crucial network of type IV collagen molecules that is necessary to maintain normal structure and function of glomerular capillary walls. The type IV collagen network in the GBM is an assembly of three distinct peptide chains, and each of these peptide chains is encoded by separate gene. A mutation in any one of these three genes can disrupt the molecular integrity of the entire collagen IV network in the GBM and cause progressive renal damage that leads to kidney failure. One of these genes (*COL4A5*) is located on the X-chromosome in both dogs and humans, and when mutations occur in this gene, the disease has an X-linked mode of inheritance in both species. The other two genes (*COL4A3* and *COL4A4*) are located on autosomes (human chromosome 2, and canine chromosome 25), and when a mutation in one of these two genes is responsible, the disease has an autosomal (usually autosomal recessive) mode of inheritance. In Cocker Spaniels, the disease is inherited as an autosomal recessive trait and the peptide products of *COL4A3* and *COL4A4* are completely absent from the GBM (see below). Therefore, *COL4A3* and *COL4A4* are the candidate genes in which the causative mutation for FN in Cocker Spaniels is likely to exist, but the mutation that causes the disease has not yet been identified.

### **Clinical signs**

Dogs with FN develop chronic renal failure, usually while they are between 6 months and 2 years of age. The clinical signs associated with chronic renal failure caused by FN are the same as those associated with chronic renal failure due to any other cause. Clinical signs that are often observed include excessive water consumption (polydipsia), excessive urine volume (polyuria), reduced growth rate or weight loss, poor quality hair coat, reduced appetite, and vomiting. Such signs can develop insidiously and escape recognition until the degree of renal failure is so severe that overt uremia supervenes. At this late stage of the disease, physical examination findings may include thin body condition, dehydration, pallor of mucous membranes, uremic breath odor, and oral ulcerations. Alternatively, especially at earlier stages of the disease, physical exam findings may be normal.

### **Laboratory features**

When dogs with FN develop renal failure, serum chemistry test results will be abnormal in proportion to the severity of renal failure. Expected abnormalities include azotemia (increased BUN and creatinine concentrations), hyperphosphatemia, and metabolic acidosis. Hematology test results usually show some degree of anemia. Urinalysis findings for dogs in renal failure invariably include evidence of impaired urine concentrating ability (inappropriately low urine specific gravity). All of the laboratory test abnormalities mentioned so far might be associated

with chronic renal failure due to any cause; they do not help to differentiate FN from any other potential cause of renal failure.

Because FN is a glomerular disease, affected dogs invariably exhibit proteinuria by the time that any clinical illness that can be attributed to FN first develops. Indeed, as renal disease evolves in dogs that have FN, proteinuria always is the first abnormality that can be detected by non-invasive testing. Of course, proteinuria can be associated with a variety of different urinary tract disorders (*e.g.*, bacterial urinary tract infection, urolithiasis, etc.), so an appropriate clinical investigation must be conducted to exclude such other conditions. Nonetheless, persistent renal proteinuria that is not otherwise explained in a young (3- to 12-month-old) Cocker Spaniel most often proves to be due to FN. If discovered very early in the course of disease, the magnitude of proteinuria may be mild, but it typically becomes marked within a month or two. Urine protein-to-creatinine (UPC) ratios commonly are in the 5-10 range, and occasionally are greater than 10. If detected early, proteinuria may be observed while urine concentrating ability is well preserved (*i.e.*, urine specific gravity is still high) and serum creatinine concentrations are still within the normal reference range. However, with continued monitoring, dogs with FN will have persistent proteinuria, will subsequently lose their urine concentrating ability, and will gradually develop increasing serum creatinine concentrations. If FN has progressed to renal failure by the time that laboratory tests are performed, the findings will include marked proteinuria and isosthenuria, as well as the hematologic and serum chemistry abnormalities expected with the severity of renal failure that then exists.

### **Treatment**

The renal disease caused by FN invariably is progressive and ultimately fatal; however, the rate of disease progression observed in affected dogs is more rapid in some individuals than in others. Additionally, certain treatments (feeding a diet formulated for dogs with renal failure and administering an angiotensin converting-enzyme inhibitor such as enalapril or benazepril) may slow the rate of renal disease progression somewhat. Even if they are effective, however, such therapeutic efforts generally forestall the development of terminal renal failure by several weeks or a few months at best. Moreover, the rate of renal disease progression usually is fairly rapid during the late stages of the disorder. Once an affected dog develops moderate azotemia, the disease typically progresses to the terminal stage of renal failure within 3 to 6 more weeks.

### **Pathologic features**

When examined by light microscopy, kidneys from dogs with FN have a constellation of abnormalities that are indicative of progressive glomerular disease. Severity of the abnormalities depends on the stage of the disease that was present when the tissue was obtained, but the lesions are non-specific. That is, at the light microscopic level, the kidney changes that are produced by FN are similar to those of other glomerular disorders in dogs. The distinctive abnormalities that set FN apart from other glomerular renal diseases are seen only with immunostaining techniques and using transmission electron microscopy.

Immunostaining of kidney with individual antibodies that specifically recognize and bind to each of the different type IV collagen peptides can be used to identify the collagen content of all renal basement membranes. Normal GBM stains strongly positive for the  $\alpha$ 3 ( $\alpha$ 3) chain, the  $\alpha$ 4 chain, and the  $\alpha$ 5 chain of type IV collagen. This staining pattern reflects presence of the normal  $\alpha$ 3- $\alpha$ 4- $\alpha$ 5 chain network that is required for long-term maintenance of GBM structure and function. In Cocker Spaniels with FN, however, staining for  $\alpha$ 3 and  $\alpha$ 4 chains is totally negative in all basement membranes where they are normally present, including the GBM. In addition,

GBM staining for  $\alpha 5$  chains is greatly reduced, and there is greatly increased staining for 3 other chains ( $\alpha 1$ ,  $\alpha 2$ , and  $\alpha 6$ ) that normally are not prominent components of the GBM. This abnormal staining pattern reflects absence of the  $\alpha 3$ - $\alpha 4$ - $\alpha 5$  collagen network from the GBM, which instead contains networks composed of  $\alpha 1$ - $\alpha 1$ - $\alpha 2$  and  $\alpha 5$ - $\alpha 5$ - $\alpha 6$  chains. These substitute type IV collagen networks do not function properly (*i.e.*, they do not function like the  $\alpha 3$ - $\alpha 4$ - $\alpha 5$  network), and as a result of this abnormality, the processes of renal injury that ultimately produce kidney failure are set into motion. This abnormal pattern of immunostaining of renal basement membranes for type IV collagen  $\alpha$ -chains is very distinctive of FN in Cocker Spaniels and can be demonstrated at all stages of the disease (*i.e.*, from birth onward).

Despite the abnormal type IV collagen content of certain renal basement membranes, the kidneys of dogs with FN initially develop normally and function properly. Within a few months, however, because the  $\alpha 3$ - $\alpha 4$ - $\alpha 5$  network is missing, the ultrastructure of the GBM, which can be seen only by using electron microscopy, starts changing. This is the first structural change in the kidneys that occurs, and it precedes any change in the kidneys that can be seen with routine light microscopy. The ultrastructural GBM changes are at first focal (*i.e.*, present in some sites but not others) and mild, but they gradually become more extensive and severe throughout the course of the disease. The ultrastructural GBM changes also are very distinctive of this disease, especially once they have become widespread and at least moderately severe. The GBM becomes greatly thickened and exhibits multilaminar splitting and fragmentation of its internal structure. These GBM changes are associated with other changes in the glomerular capillary wall that alter its filtration properties, which causes the proteinuria to develop.

As the disease progresses, light microscopic lesions develop both in the glomeruli and in the tubulointerstitium. The morphologic glomerular lesions are those of a membranoproliferative disorder progressing to glomerular sclerosis and obsolescence with periglomerular fibrosis. The tubulointerstitial changes, which always are prominent when renal failure has developed, include extensive tubular degeneration, dilation, and atrophy together with interstitial inflammation and fibrosis. As emphasized previously, these light microscopic lesions are non-specific; they are not sufficient by themselves for definitive diagnosis of FN.

### **Definitive diagnosis**

In conjunction with a well documented clinical course of illness that is compatible with FN (and appropriate clinical investigations that failed to identify other causes of renal disease), light microscopic findings that are consistent with FN are sufficient for a *presumptive* diagnosis of the condition. Nevertheless, *definitive* diagnosis of FN always rests on demonstrating at least one, and preferably both, of the distinctive abnormalities that uniquely distinguish FN from all other causes of renal disease. Therefore, the definitive diagnosis of FN always requires special studies (*i.e.*, electron microscopy, or immunostaining, or both) of kidney from the affected dog. Availability of centers that can perform such evaluation is limited, but even more importantly, ability to perform (and interpret) such studies depends on having suitable tissue specimens for examination, even if a center that can perform such studies is identified. As a rule, tissues that are routinely fixed in 10% formalin, the usual fixative that is used for light microscopic studies, are not satisfactory for electron microscopy or immunostaining. Consequently, obtaining tissue specimens that will be suitable for such studies typically requires prior preparation and planning to become familiar with the appropriate procedures and assemble the needed materials.

**Appendix** - Guidelines for collecting renal tissue specimens for pathologic studies from dogs that are suspected to have FN

1. Time matters, especially for the electron microscopy specimens. Post-mortem collection of renal tissue specimens should be accomplished immediately after the dog's death or euthanasia. Renal biopsy specimens also should be processed immediately.
2. Another crucial issue is that the tissue specimens for electron microscopy and immunostaining must contain glomeruli; that is, they must be samples of renal cortex.
3. The key factor for satisfactory electron microscopic studies is complete, rapid fixation of the tissue (ultrastructural features the glomerular cells and GBM begin to deteriorate within a few minutes, and the purpose of "fixation" is to halt this process). Optimum fixation is provided by special fixatives (*e.g.*, 2.5% or 3.0% glutaraldehyde in a phosphate buffer) that are intended for electron microscopy. Fixative solutions for electron microscopy must be kept refrigerated and generally have a short shelf-life; we routinely prepare a fresh supply of fixative every 30 days. Besides using an appropriate fixative solution, good fixation is promoted by cutting the tissue specimen in to sufficiently small pieces (1 mm cubes), but this must be done without crushing the specimen. We usually place a piece of renal cortex (about 5-6 mm cube) on a clean glass slide in a small puddle of the fixative (taken from the "EM specimen vial" with a pipette) and then use a fresh single-edge razor (or scalpel) blade to cut the tissue in half, then each piece in half again, and so on until all the pieces are about 1 mm cubes. These are then put in the "EM specimen vial" (which is promptly refrigerated again).

When a fixative that is intended for electron microscopy is not available, the next best fixative solution to use is 10% formalin (*i.e.*, as for light microscopy). Fixation attained in this way can be satisfactory, but starting immediately (*i.e.*, with tissue that is as fresh as possible) and making the pieces sufficiently small is all the more important because formalin fixation proceeds more slowly than fixation with conventional electron microscopy fixatives.

4. Immunostaining is performed using unfixed tissue specimens that are snap-frozen (with liquid nitrogen) and kept frozen thereafter. To avoid having to ship frozen specimens, we use Michel's Transport Medium (a non-fixative solution formulated for this purpose) to preserve the specimen until it gets to our laboratory; we then freeze it. Michel's Transport Media also has a short shelf-life and must be kept refrigerated (during storage and use), but it does keep the tissue suitable for eventual immunostaining for up to 2-3 days (*i.e.*, long enough for it to be delivered to our lab).
5. We also always want to receive conventional formalin-fixed specimens of kidney for routine light microscopic examinations as well as the special studies.

Note: the tissue specimens described above are used for diagnostic evaluations. For our genetic studies of FN, we also want a blood sample (in a "purple-top" EDTA tube; for DNA) and some pieces of renal cortex in placed in RNALater™ (for RNA isolation). Within the United States, because many of the component materials have a short shelf-life and must be kept refrigerated, we prepare and ship kits containing all the necessary materials and instructions to veterinarians "upon request." Using courier services (*e.g.*, FedEx) for overnight delivery, we can provide such a kit in a timely manner with as little as 24-36 hours of prior notice (barring weekends).

### **Selected references:**

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