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Commentary

Alport Syndrome with Diffuse Leiomyomatosis

When and When Not?

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The 1990s have been an exciting and productive decade for the molecular dissection of the etiology of Alport syndrome. Alport syndrome is a hereditary glomerulonephritis accompanied usually by sensorineural deafness, frequently by ocular abnormalities, and rarely by diffuse leiomyomatosis (DL), which is characterized by benign nodular smooth muscle tumors of esophagus, tracheobronchial tree, and genital tract. The primary mode of inheritance of Alport syndrome is X-linked-dominant, though there are also autosomal-recessive and autosomal-dominant forms, and its overall prevalence is estimated to be 1 in 5000. The only treatments for the nephropathy at end stage are dialysis and renal transplantation.[1–6]

Alport syndrome is a basement membrane disease involving type IV collagen. Collagen IV is a major component of all basement membranes. It is composed of α chains that trimerize to form long triple helical protomers. Protomers are secreted by cells and associate with each other in the extracellular matrix to form a chicken-wire-like network.[7] This serves as the scaffold for assembly of the basement membrane, which also contains laminin, entactin/nidogen, and sulfated proteoglycans.[8,9]

There are six genetically distinct collagen IV α chains, α1(IV)-α6(IV). The collagen α1(IV) and α2(IV) chains are the classical chains and are essentially ubiquitous in basement membranes. Mutations in *COL4A1* and *COL4A2* have not been found in mammals and would likely be embryonically lethal. In contrast, the underlying genetic defect in Alport syndrome is a mutation in any one of three genes encoding what have been termed novel type IV collagen chains. These chains have a restricted tissue distribution. Importantly, they are all major components of the glomerular basement membrane (GBM), which is characteristically thinned, thickened, and split in Alport syndrome. X-linked Alport syndrome is caused by mutations in the collagen α5(IV) chain gene *COL4A5*, and mutations in *COL4A3* and *COL4A4,* which are linked head-to-head on chromosome 2, are responsible for the autosomal forms of the disease.[10–12]

A molecular hallmark of the severe forms of Alport syndrome is that mutations affecting only one of the *COL4A3-COL4A5* genes result in the absence all three gene products from the GBM. This has been used as circumstantial evidence to suggest a model in which the α3-α5(IV) chains coassemble in a manner that requires all three chains.[13] In this model, the nonmutated genes would be transcribed and translated normally, but the chains they encode would be degraded on failing to assemble due to the absence of a normal third chain. This model is attractive, because it is consistent with the inherent trimeric structure of collagen IV protomers. Alternatively, the defect in assembly could be at the level of protomer:protomer interactions in the extracellular matrix.

Good evidence for transcriptional down-regulation of the nonmutated genes in a canine model of Alport syndrome has been presented. These data show that the lack of collagen α5(IV) protein due to *COL4A5* mutation was associated with a decrease in α3(IV) and α4(IV) steady-state mRNA levels.[14] This could explain in part the absence of the α3(IV) and α4(IV) chains in mutant dog kidney basement membranes. However, RNA studies in humans and in the two mouse models of Alport syndrome did not find such a down-regulation in steady-state mRNA levels and thus do not support such a transcriptional mechanism.[15–17]

The collagen α6(IV) chain is unique in that it has a restricted tissue distribution but is not deposited in the GBM. It is found in basement membranes associated with Bowman's capsule, epidermis, and a subset of smooth muscle cells.[18,19] Consistent with its absence from GBM, mutations that affect only *COL4A6* have not been found in Alport patients.[20] However, *COL4A6* is located on the X chromosome head-to-head with *COL4A5* and some *COL4A5* deletion mutations that cause Alport syndrome extend into *COL4A6*.[21–23] Thus,

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the 5′ ends of both genes are affected. Cases of Alport syndrome associated with diffuse leiomyomatosis always fall into this category, but the extent of the deletion into *COL4A6* is limited to the alternative exons 1 and 1′, intron 1, exon 2, and part of the very large intron 2. Interestingly, if the deletion extends into exon 3, then diffuse leiomyomatosis is not observed.[22,23]

This leads to the question of how and why some deletions that affect *COL4A6* result in diffuse leiomyomatosis, whereas the most extensive ones do not. It has been hypothesized that the more restricted deletions may allow production of a truncated α6(IV) protein in smooth muscle that might be capable of aberrant signaling and lead to the observed benign tumors.[22] However, no stable integration of any α6(IV) protein into esophageal tumor basement membranes from appropriate patients was observed.[24] Another possibility is that there is a gene, which may or may not encode a protein, embedded in the large second intron of *COL4A6* that is somehow transformed into a dominant promoter of smooth muscle cell proliferation by the deletions that cause Alport syndrome with diffuse leiomyomatosis.[22] Further studies of the ~140-kb second intron of *COL4A6* will be necessary to test this hypothesis.

COL4A5-specific mutations lead to the absence of collagen α6(IV) in renal and epidermal basement membranes,[18,19,25] suggesting that the α6(IV) chain cannot assemble into these basement membranes without the α5(IV) chain. One important issue that has not been addressed is the status of collagen α6(IV) protein in the smooth muscle basement membranes of such Alport patients, who do not have deletions extending into *COL4A6* and who do not develop leiomyomata. The formal possibility exists that, despite its absence from kidney and skin basement membranes, these patients maintain a somewhat normal complement of α6(IV) protein in their smooth muscle basement membranes. This might play some role in preventing overproliferation of smooth muscle cells. However, if true, then it would be difficult to explain the absence of tumors when *COL4A6* deletions extend into exon 3. Nevertheless, whether *COL4A5*-specific mutations lead to an absence of α6(IV) in smooth muscle basement membranes is certainly worth investigating.

The article by Zheng et al[26] published in this issue of *The Americal Journal of Pathology* finally addresses this and other important points using a canine model of X-linked Alport syndrome. Paul Thorner and colleagues have previously studied this family of Samoyed dogs in depth; they have identified a single base non-sense mutation in *COL4A5* and have shown that the affected dogs exhibit many of the characteristics observed in human Alport syndrome.[27–30] In this issue of the *Journal* they report the cloning and sequencing of DNA adjacent to the 5′ end of canine *COL4A5* and show that dog has a *COL4A6* gene with many similarities to the human gene, including the tightly linked, head-to-head arrangement with *COL4A5*. This is the first cross-species comparison of this region, and it shows that although exon 1 is very conserved between human and dog, exon 1′ is not. The authors rightly question the functionality of this exon in dog. Indeed, by Northern blot analysis, they show that *COL4A6* mRNAs from bladder smooth muscle contain exon 1 but not exon 1′. The authors use immunohistochemistry to show that the collagen α6(IV) chain is present in bladder smooth muscle basement membranes from a normal dog but is completely absent from the *COL4A5* mutant dog smooth muscle. Moreover, despite the absence of α6(IV) protein, α6(IV) mRNA levels in bladder smooth muscle are nearly normal. Finally, leiomyomata have never been observed in this family of dogs.

These results reveal important new information regarding the biology of type IV collagen and the etiology of Alport syndrome with diffuse leiomyomatosis. First, a point mutation in *COL4A5* is sufficient to prevent incorporation of the collagen α6(IV) chain into smooth muscle basement membranes, independent of a reduction in α6(IV) mRNA levels. This provides further evidence for requisite coassembly of the α5 and α6(IV) chains, in agreement with the observed absence of α6(IV) from renal and epidermal basement membranes in Alport patients with *COL4A5*-specific mutations.[18,19,25] However, it contrasts with the transcriptional mechanisms previously proposed as negative regulators of expression of the α3 and α4(IV) chains in *COL4A5* mutant dog kidney.[14]

Second, the mere absence of α6(IV) from dog smooth muscle is not sufficient to cause diffuse leiomyomatosis. By analogy, based on these studies of dog, human Alport patients with *COL4A5* mutations would lack the α6(IV) chain in smooth muscle, but only those with the additional appropriate *COL4A6* deletions would develop leiomyomata. Thus, these deletions are likely affecting something other than expression of α6(IV) and its incorporation into basement membranes. Determining what this something really is will solve an important mystery and could force revisions in our understanding of gene structure, regulation of cell proliferation, and development of tumors and perhaps cancer.

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