Animal models of Alport syndrome

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Introduction

The last decade of the twentieth century was a very productive period for the study of Alport syndrome. Alport syndrome was shown to result from mutations in certain members of the type IV collagen family of proteins, the \( \alpha_3(IV) \), \( \alpha_4(IV) \), and \( \alpha_5(IV) \) chains. Several hundred different mutations in the \( \text{COL4A5} \) gene, which encodes the \( \alpha_5(IV) \) chain, were described in patients with X-linked Alport syndrome [1]. A few dozen mutations were found in the \( \text{COL4A3} \) and \( \text{COL4A4} \) genes, which respectively encode the \( \alpha_3(IV) \) and \( \alpha_4(IV) \) chains, in patients with autosomal recessive Alport syndrome [2,3]. Autosomal dominant Alport syndrome, due to heterozygous mutations in \( \text{COL4A3} \) or \( \text{COL4A4} \), was established as an entity, and distinguished from Fechtner and Epstein syndromes, which arise from mutations in a non-collagen locus, \( \text{MYH9} \) [4–7]. Investigators established that mutations in the \( \alpha_3(IV) \), \( \alpha_4(IV) \), or \( \alpha_5(IV) \) chain typically prevent expression of any of these proteins in glomerular basement membranes (GBM), because these chains must interact with each other in networks in order to be deposited in the GBM [8]. These chains are replaced in the GBM by \( \alpha_1(IV) \) and \( \alpha_2(IV) \) chains, which normally disappear, for the most part, from the GBM during the capillary loop stage of glomerular maturation [9–11]. Other extracellular matrix proteins, such as types V and VI collagen and the laminin \( \alpha_2 \) chain, are aberrantly expressed in the Alport GBM [9,12].

Despite these advances, key questions about Alport syndrome remain. Except perhaps for anterior lenticonus, for which it can be plausibly argued that reduced tensile strength of the lens capsule basement membrane prevents maintenance of normal lens shape, we have very little understanding of the pathogenesis of the extrarenal manifestations of Alport syndrome, in particular sensorineural deafness. We do not know how the Alport GBM loses its permselectivity, or why Alport kidneys fail. Of greatest importance, we do not have treatments of proven efficacy, and the nature of the disease—its relative rarity, its slow progression, and the strong influence of genotype on the severity of the Alport phenotype—will complicate efforts to conduct randomized clinical trials.

Animal models of genetic disorders provide opportunities for investigating both pathogenesis and treatment of disease. Fortunately, several excellent animal models of Alport syndrome have been developed (Table 1). Thus far, these models are limited to spontaneous Alport syndrome in dogs and transgenic models of Alport syndrome created in mice. This review provides a brief discussion of these models and their applicability to investigations of the pathogenesis and treatment of Alport syndrome, and of the biology of type IV collagen.

X-linked Alport syndrome (XLAS)

About 80% of patients with Alport syndrome have the X-linked form of the disease. Two canine models of XLAS have been identified. The first of these was discovered in a strain of Samoyed dogs, and was found to result from a G to T substitution in exon 35 (of 51) of \( \text{COL4A5} \), changing a glycine codon to a stop codon [13,14]. The second, found in a kindred of mixed-breed dogs from Navasota, Texas [15], arises from a 10-bp deletion in exon 9 of \( \text{COL4A5} \) that creates a frameshift and a stop codon in exon 10 (Cox M., Lees G., Kashtan C., Murphy K., submitted for publication). Affected animals in both models display ultrastructural changes in the GBM, and alterations in the GBM expression of collagen IV \( \alpha \) chains, that are indistinguishable from those found in human Alport patients. Overt proteinuria appears in affected males during the first 6 months of life, and uraemia by 12–18 months. Affected males do not have clinically detectable deafness or ocular abnormalities.
At this time, there is no reported murine model of XLAS. A murine XLAS model would be valuable because XLAS is the predominant human form of the disease, and would facilitate investigation of the role of X-inactivation in determining outcome in female heterozygotes.

Autosomal recessive Alport syndrome (ARAS)

ARAS accounts for about 15% of patients with the disease. The existence of an inherited nephropathy in English cocker spaniels was known for many years, and in the 1980s this was shown to be a progressive glomerular disorder with autosomal recessive inheritance [16,17]. GBM of affected dogs showed changes similar to Alport syndrome by electron microscopy, and was found to lack the \( \alpha_3(IV) \) and \( \alpha_4(IV) \) chains [18,19]. Expression of the \( \alpha_5(IV) \) chain in the GBM is reduced, but not absent. The mutation responsible for ARAS in English cocker spaniels has not yet been identified, although efforts to sequence the \( \text{COL4A3} \) and \( \text{COL4A4} \) genes in normal and affected dogs are under way.

Several transgenic models of ARAS have been developed. Two of these models involve knockouts of the murine \( \text{col4a3} \) gene [20,21]. In the third model, the proximal portions of both the \( \text{col4a3} \) and the \( \text{col4a4} \) gene were deleted (this is possibly because these genes are located adjacent to each other, in a 5' to 5' orientation) [22]. The GBM of homozygous ARAS mice lacks the \( \alpha_3(IV) \), \( \alpha_4(IV) \), and \( \alpha_5(IV) \) chains, and by electron microscopy shows the basket-weave transformation characteristic of human Alport syndrome [20–22]. Overt proteinuria and uraemia develop within a few months of birth in murine ARAS (Table 1).

Autosomal dominant Alport syndrome (ADAS)

Hood and colleagues [23,24] have described an autosomal dominant nephropathy in bull terriers with GBM changes similar to those occurring in Alport syndrome. The mutation responsible for this disorder has yet to be identified. The GBM of affected dogs does not exhibit abnormal collagen IV \( \alpha \)-chain expression, but this does not exclude a heterozygous mutation in \( \text{COL4A3} \) or \( \text{COL4A4} \) as the cause of the disease.

Research applications of animal models of Alport syndrome

Both canine and murine models of Alport syndrome are associated with advantages and disadvantages. Canine breeding programmes are expensive to maintain, and gestation periods are relatively long. An advantage of canine models is the larger size of the animal, which will facilitate development of gene transfer protocols for the treatment of Alport syndrome that are technically applicable to the human disease [25].

Murine models have the advantage of a short gestation period. The murine forms of Alport syndrome progress very rapidly (Table 1). On the one hand, this minimizes the time needed to determine the effect of a therapeutic intervention. On the other hand, while prolongation of survival of a few weeks might reach statistical significance in a murine model, such an effect might be difficult to confirm in human subjects. Some apparent therapeutic effects may be strain-specific, due to the influence of a predominant modifying gene allele (see Note added in proof).

Animal models of Alport syndrome will be of great value to investigators interested in the functions of the \( \alpha_3(IV) \)–\( \alpha_6(IV) \) chains. The \( \alpha_1(IV) \) and \( \alpha_2(IV) \) chains have been essential components of multi-cellular organisms for hundreds of millions of years [26]. The \( \alpha_3(IV) \)–\( \alpha_6(IV) \) chains are relatively recent additions to the mammalian protein repertoire, arising from duplication and modification of the \( \alpha_1(IV) \) and \( \alpha_2(IV) \) genes [27]. What purposes do the \( \alpha_3(IV) \)–\( \alpha_6(IV) \) chains serve? They are clearly not required for fetal survival or for the formation of kidneys, ears and eyes that are, from birth and for a substantial time thereafter, essentially normal in structure and function. The \( \alpha_3(IV) \)–\( \alpha_4(IV) \)–\( \alpha_5(IV) \) chains may protect GBM from proteolysis, since GBM composed of these chains is more resistant

### Table 1. Animal models of Alport syndrome

<table>
<thead>
<tr>
<th>Model</th>
<th>Genetics</th>
<th>Mutation</th>
<th>Onset of proteinuria</th>
<th>Timing of ESRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine Samoyed</td>
<td>X-linked</td>
<td>G to T in exon 35 of COL4A5, creating premature stop</td>
<td>4 months</td>
<td>8–10 months</td>
</tr>
<tr>
<td>Navasota</td>
<td>X-linked</td>
<td>10-bp deletion in exon 9 shift and premature stop</td>
<td>3–4 months</td>
<td>10–15 months</td>
</tr>
<tr>
<td>English cocker spaniel</td>
<td>Autosomal recessive</td>
<td></td>
<td>5–8 months</td>
<td>12–18 months</td>
</tr>
<tr>
<td>Bull terrier</td>
<td>Autosomal dominant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Murine COL4A3 Knockouts</td>
<td>Autosomal recessive</td>
<td>COL4A3 deactivated</td>
<td>2–3 months</td>
<td>3–4 months</td>
</tr>
<tr>
<td>tg/tg mice</td>
<td>Autosomal recessive</td>
<td>COL4A3 &amp; COL4A4 deactivated</td>
<td>2 weeks</td>
<td>8–12 weeks</td>
</tr>
</tbody>
</table>

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to proteolytic degradation than GBM containing only z1(IV)–z2(IV) chains [10]. The supramolecular assembly of z3(IV)–z4(IV)–z5(IV) protomers is stabilized by disulphide bonds not present in z1(IV)–z2(IV) networks, which may confer greater mechanical stability [8]. Adhesion of glomerular epithelial cells to GBM appears to be mediated, at least in part, by interaction between the z3/β1 integrin, the major integrin expressed by these cells, and the z3(IV) chain [28]. Podocytes may be able to adhere to an z3(IV)-deficient GBM, but maintenance of the fully differentiated podocyte phenotype may require interaction with the z3(IV) chain.

In summary, animal models of Alport syndrome offer excellent opportunities for investigation of pathogenesis and treatment of the disease, and for answering basic questions about the roles of type IV collagen chains in the biology of the kidney, cochlea, eye, and other tissues.

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Note added in proof

Andrews et al. [29] have reported that quantitative trait loci influence disease progression in murine Alport syndrome. The Navasota COL4A5 mutation was presented in abstract by Melissa Cox at the Keystone Symposium ‘Genotype to Phenotype Focus on Disease’, Santa Fe, New Mexico, February 19–24, 2002.

References