Alport syndrome and the X chromosome: implications of a diagnosis of Alport syndrome in females

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End-stage renal disease in women with Alport syndrome

In his 1927 report on ‘hereditary familial congenital haemorrhagic nephritis’, A. Cecil Alport [1] noted that the ‘male members of a family tend to develop nephritis and deafness and do not as a rule survive’.
while ‘the females have deafness and haematuria and live to old age’. This characterization of the impact of gender on the outcome of Alport syndrome has been conventional wisdom for nearly 80 years. However, the family described by Alport included a female with haematuria and deafness who died at 24 years of age. Alport may have considered this woman an exception, but with the passage of time it has become clear that women with Alport syndrome are indeed at risk for progression to end-stage renal disease (ESRD).

Descriptions of Alport kindreds have often included affected women who developed uraemia. For example, Crawford and Toghill [2] described sisters in a large Alport family who died of uraemia at ages 43 and 55. Ferguson and Rance [3] collected seven Alport families with 16 affected females, two of whom died of uraemia. Gaboardi et al. [4] reported on 19 Alport families; 11 of 46 affected females required dialysis or died from renal failure during the fourth to sixth decades of life. In each of these studies, the families exhibited a dominant pattern of disease transmission consistent with X-linked inheritance.

The risk of ESRD in women with X-linked Alport syndrome was clarified by a recent study by Jais et al. [5] of 288 affected women from 195 families. ESRD had developed in 51 women (18%) and another 34 patients (12%) had chronic renal failure; these outcomes are comparable with those reported by Gaboardi et al. [4] in which 24% (11/46) women developed uraemia. Of those women who developed ESRD, it occurred between 19 and 30 years in 28%, between 31 and 40 years in 31% and after 41 years in 41% [5]. Approximately one-third of women greater than age 60 who were regularly followed had developed ESRD [5].

Grünfeld [6] identified risk factors for the development of ESRD in a retrospective analysis of 36 women with Alport syndrome, 14 of whom progressed to ESRD. Features suggestive of progressive disease in females included a history of gross haematuria in childhood, nephrotic syndrome and the presence of diffuse thickening of glomerular basement membranes by electron microscopy. Jais et al. [5] found that development of proteinuria conferred a significantly increased risk for development of ESRD in women with Alport syndrome. Among women with proteinuria, ESRD occurred in 20% by age 40 and in 30% by age 60, while no woman developed ESRD in the absence of proteinuria. Proteinuria was found in 75% of women who were tested.

These observations have important implications for clinicians providing care for families with Alport syndrome and for the selection of living donors for Alport patients facing renal transplantation:

(i) Females with Alport syndrome should undergo regular prospective examination. The appearance of proteinuria should be considered ominous. Although data demonstrating that angiotensin blockade preserves renal function in Alport females with proteinuria is lacking, treatment should be considered, once overt proteinuria has developed.

(ii) Clinicians caring for boys with Alport syndrome, or girls with Alport syndrome whose fathers are unaffected, should attempt to determine whether their mothers know their clinical status and should recommend evaluation by a nephrologist if they are not already being followed.

(iii) Regarding women with Alport syndrome serving as kidney donors, the most conservative position would be to discourage kidney donation by these women, even when urine protein excretion is normal. Current information indicates that most women with Alport syndrome will develop proteinuria and that proteinuria significantly increases the risk of ESRD [5]. Since we currently cannot identify those women who will never develop proteinuria, we must assume that any woman with Alport syndrome is significantly more likely to develop ESRD sometime in her life, compared with the normal population. Women who are asymptomatic carriers of Alport syndrome, i.e. those without haematuria who represent about 5% of women with X-linked disease [5], would escape this prohibition, as their risk of ESRD is presumably very low.

A somewhat more relaxed approach would allow kidney donation by X-linked Alport syndrome (XLAS) females who are over a certain age, e.g. 45–50 years, and still exhibit normal urine protein excretion and hearing, as suggested in a recent review of renal transplantation in Alport syndrome [7]. Even under these circumstances, women with XLAS should be considered donors of last resort, to be considered after other potential donors have been excluded.

Genetic aspects of Alport syndrome in women

There are three genetic forms of Alport syndrome. XLAS arises from mutations in the COL4A5 gene and accounts for about 80% of families with the disease. About 15% of families have autosomal recessive Alport syndrome (ARAS) due to mutations in both alleles of the COL4A3 or COL4A4 gene. The autosomal dominant form of Alport syndrome (ADAS) occurs in about 5% of families, and is caused by heterozygous mutation in COL4A3 or COL4A4 [8].

For women, the genetic type of Alport syndrome has major prognostic and reproductive implications. The risk of ESRD in women with ARAS is probably 100%, with most reaching terminal renal failure by the fourth decade of life. On the other hand, a woman with ARAS has an extremely low risk of transmitting the disease to her offspring. As noted above, a substantial minority of women with XLAS will develop ESRD at some point during their lives [5]. Each child of a woman with XLAS has a 50% likelihood of being affected and all affected male offspring will develop...
Table 1. Hypothetical correlation between type IV collagen genotype, protein expression, glomerular basement membrane (GBM) structure and renal phenotype.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Synthesis of α3 α4 α5(IV) content of GBM</th>
<th>GBM structure</th>
<th>Renal phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>100%</td>
<td>Normal width &amp; structure</td>
<td>None</td>
</tr>
<tr>
<td>Heterozygous mutation in COL4A3 or COL4A4 Mutations in both alleles of COL4A3 or COL4A4</td>
<td>50%</td>
<td>Uniformly thin</td>
<td>Hematuria</td>
</tr>
<tr>
<td>Heterozygous mutation in COL4A5</td>
<td>Two cell populations; wild-type cells produce normal amounts of α3α4α5(IV); mutant cells produce no α3α4α5(IV)</td>
<td>Two GBM species; wild-type GBM has normal α3α4α5(IV) content; mutant GBM has no α3α4α5(IV)</td>
<td>Mix of normal, thin &amp; thick (lamellated)</td>
</tr>
<tr>
<td>Hemizygous mutation in COL4A5</td>
<td>0%</td>
<td>Thin (early); thick &amp; lamellated (later)</td>
<td>Hematuria proteinuria ESRD</td>
</tr>
</tbody>
</table>

ESRD eventually. In ADAS, ESRD occurs relatively late in life [9] and the risk of disease transmission is 50% with each pregnancy.

Renal outcomes in women who are heterozygous for a mutation in COL4A5 are markedly different from outcomes in women and men who have heterozygous mutations in COL4A3 or COL4A4. Heterozygotes for COL4A3 and COL4A4 mutations may be asymptomatic or exhibit microscopic haematuria [10,11]. Those with haematuria typically exhibit thin glomerular basement membranes by renal biopsy and development of proteinuria and renal insufficiency is rare [12].

Table 1 attempts to explain the difference between COL4A5 heterozygotes and COL4A3/COL4A4 heterozygotes on the basis of the impact these mutations may have on glomerular distribution of the type IV collagen network formed by α3(IV), α4(IV) and α5(IV) chains. There are two type IV collagen networks in glomerular basement membranes. One is composed of α1α2α3α5(IV) trimers synthesized by the COL4A1 and COL4A2 genes and the other is made up of α3α4α5(IV) trimers generated by the COL4A3, COL4A4 and COL4A5 genes [13]. In the typical male with XLAS, glomerular basement membranes are completely lacking in the α3α4α5(IV) network but exhibit increased amounts of the α1α2α3α5(IV) network [14,15]. Since normal males have only one COL4A5 allele, it is clear that a single normal COL4A5 allele provides sufficient α5(IV) substrate for α3α4α5(IV) network formation. In contrast, a single mutant COL4A5 allele prevents formation of the α3α4α5(IV) network, despite the presence of normal COL4A3 and COL4A4 alleles. In females with XLAS, GBM-producing cells, presumably podocytes, will have either an active normal COL4A5 allele and produce normal α3α4α5(IV) trimers, or an active mutant COL4A5 allele, preventing formation of normal α3α4α5(IV) trimers. The glomeruli of a female with XLAS would be expected to exhibit a mixture of normal, α3α4α5(IV)-positive GBM and abnormal, α3α4α5(IV)-negative GBM.

There are several possible explanations for the phenotypic variability of XLAS in women. Phenotypic severity could reflect the relative activities of the mutant and normal COL4A5 alleles, as determined by random or non-random X-inactivation. The COL4A5 genotype could influence phenotype in females as it does in males [16], although the available evidence indicates that genotype and phenotype are not clearly correlated in females with XLAS [5].

It is widely assumed that the variability in renal outcomes in women with XLAS is a consequence of the variable balance of wild-type and mutant alleles resulting from random X-chromosome inactivation. In fact, studies of this question have thus far failed to provide definitive evidence that X-chromosome inactivation balance determines renal prognosis in women with XLAS. Vetrie and colleagues [17] studied X-chromosome inactivation patterns in peripheral blood cells from 30 females with XLAS, including 25 females with normal renal function and five with impaired renal function. Their findings provided no evidence for selection against the mutant X chromosome (non-random inactivation) or for the hypothesis that increased severity of disease is associated with chance inactivation of a high proportion of normal X chromosomes.

This admirable study did have limitations. The study was designed to detect a correlation between a marked imbalance in X-inactivation (a ratio greater than 80:20) and a severe phenotype (renal insufficiency). A regression analysis comparing the activation ratio of the mutant and normal alleles to a quantitative measure of renal disease, such as urine protein levels or an index of phenotypic severity, like the one proposed in Table 2, might have revealed a significant correlation. The study did not specify the ages of the female subjects. Since age has a major influence on disease expression in XLAS females [5], an adjustment of data for age is likely to be an important feature of any outcome study in XLAS females.
Table 2. A possible index of phenotypic severity for females with XLAS.

<table>
<thead>
<tr>
<th></th>
<th>Points</th>
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<tbody>
<tr>
<td>Haematuria</td>
<td>1</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>1</td>
</tr>
<tr>
<td>Any</td>
<td>1</td>
</tr>
<tr>
<td>Urine protein/creatinine ratio &gt;3, or 24 h excretion &gt;3 gm</td>
<td>2</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td></td>
</tr>
<tr>
<td>GFR &lt;80 ml/min/1.73 m²</td>
<td>1</td>
</tr>
<tr>
<td>GFR &lt;50 ml/min/1.73 m²</td>
<td>2</td>
</tr>
<tr>
<td>GFR &lt;20 ml/min/1.73 m², on dialysis or transplanted</td>
<td>3</td>
</tr>
</tbody>
</table>

An asymptomatic carrier would have a score of 0.

If X-inactivation balance determines outcome in XLAS females, and if expression of the α5(IV) chain in basement membranes accurately reflects this balance, measurement of basement membrane α5(IV) might be a useful prognostic indicator in XLAS females. Two groups of investigators have studied the correlation between α5(IV) expression in epidermal basement membranes (EBM) and renal disease severity in XLAS females and published contradictory results. Nakanishi and colleagues [18] found a highly significant negative correlation between expression of the α5(IV) chain in EBM of females with XLAS, as estimated by the ratio of α5(IV)-positive EBM to total EBM, and disease severity, using urinary protein–creatinine ratio as the indicator. On the other hand, Massella and co-workers [19] found no correlation between α5(IV) expression in EBM and proteinuria in another group of XLAS females. These discrepant results do not disprove the hypothesis that X-inactivation balance determines outcome in XLAS females, but they do suggest that EBM α5(IV) expression should be used cautiously, if at all, to predict renal prognosis in girls and women with XLAS.

Interestingly, the question of the relationship between X-inactivation and disease manifestations in women with Fabry disease also remains unsettled. While Maier et al. [20] found no evidence to support such a relationship, Dobrovolny and colleagues [21] reported a statistically significant correlation between X-inactivation and clinical phenotype, using an age-weighted index of disease severity.

Females with a variety of X-linked dermatoses exhibit cutaneous mosaicism, with areas of normal skin interspersed with regions of diseased skin. The manifestations of cutaneous mosaicism often comprise a consistent pattern of lines, termed ‘Blaschko lines’, characterized by a fountain-like pattern on the skin of the back, abdominal whorls and linear stripes on the limbs [22]. Analogous manifestations of mosaicism have been described in extracutaneous structures, including the retina, lens, iris, teeth, bone and brain [23]. Preconditions for the development of these striated and mottled patterns of mosaicism include (i) establishment of genetic mosaicism in the late blastocyst, prior to embryonic development; (ii) survival and proliferation of mutation-bearing cells; (iii) limited migration of affected cells; (iv) a gene product that is not released into the circulation; (v) the gene in question cannot be located in a region of the X chromosome that escapes inactivation. Whether the tissue expression of the α5(IV) chain in XLAS heterozygotes conforms to Blaschko lines or their equivalents remains an open question. The mottled distribution of α5(IV) expression in the skin and kidney of females with XLAS (Figure 1) does appear to be consistent with X-inactivation at the late blastocyst stage of embryogenesis.

Diagnosis of Alport syndrome in girls and women

Accurate diagnosis of Alport syndrome in girls and women can be challenging, because many affected females exhibit only microscopic haematuria, and glomerular basement membrane attenuation indistinguishable from thin basement membrane nephropathy may be the sole pathological abnormality. Keys to the diagnosis of Alport syndrome in such individuals include family history and immunohistochemical analysis of type IV collagen expression in basement membranes of the skin or kidney.

Alport syndrome should be suspected in women with haematuria and a positive family history of kidney failure. A negative family history for renal failure does not, however, exclude a diagnosis of Alport syndrome. In some women with longstanding haematuria, a diagnosis of Alport syndrome is established only after the diagnosis is made in a child.

How much workup is indicated in a female with haematuria and an established family history of Alport syndrome? Although the likelihood that haematuria in such a patient results from a condition other than Alport syndrome is low, this author’s practice is to obtain a renal ultrasound in girls under 6 years of age, in order to exclude Wilms tumour. An individualized approach should be taken toward female members of Alport kindred whose haematuria is associated with atypical symptoms, such as dysuria or flank pain, or unexpectedly severe abnormalities of renal function, such as heavy proteinuria or azotaemia at a young age.

How can type IV collagen immunohistochemistry (IHC) contribute to the diagnosis of Alport syndrome in females? The utility of this methodology is based on the impact that mutations in type IV collagen loci have on expression of type IV collagen α chains in basement membranes. The effect of most COL4A5 mutations is to prevent deposition of α3α4α5(IV) trimers in basement membranes of glomeruli, Bowman’s capsules and distal tubules. Most COL4A3 mutations also prevent deposition of α5α5α6(IV) trimers in Bowman’s capsules, distal tubular basement membranes and EBM. Consequently, in about 80% of males with XLAS, immunostaining of kidneys with monospecific antibodies to the α3(IV), α4(IV) and α5(IV) chains yields entirely negative results and EBM are also negative for the α5(IV) chain.
Type IV collagen IHC is abnormal in about two-thirds of XLAS females. In kidney, glomeruli typically show segmental absence of glomerular basement membrane staining for the $\alpha_3(IV)$, $\alpha_4(IV)$ and $\alpha_5(IV)$ chains (Figure 1) [24]. Skin biopsies show interrupted staining for the $\alpha_5(IV)$ chain in EBM (Figure 1). Muda and colleagues [25] reported that three-dimensional reconstruction using confocal microscopy increased the sensitivity of $\alpha_5(IV)$ immunostaining for diagnosis of XLAS by skin biopsy.

It is necessary to enumerate some caveats regarding type IV collagen IHC for diagnosis of Alport syndrome. First, it should be clear from the frequencies of abnormal IHC cited above that a normal result cannot exclude a diagnosis of XLAS, either in males or females. Second, type IV collagen IHC is unlikely to be helpful in identifying asymptomatic carrier females (i.e. normal urinalysis), who comprise about 5% of females with XLAS [5]. The absence of haematuria in a carrier female implies a very low level of expression of the mutant $COL4A5$ allele, which would also result in minimal and likely undetectable abnormalities of basement membrane type IV collagen deposition. Third, there are instances where a $COL4A5$ mutation is associated with diminished, albeit positive, basement membrane staining for the $\alpha_3(IV)$, $\alpha_4(IV)$ and $\alpha_5(IV)$ chains. In such cases, segmental abnormalities of staining may be difficult to identify with certainty.

Females with ARAS also typically display abnormal type IV collagen IHC [26]. However, these abnormalities differ from those observed in XLAS females. The characteristics that distinguish type IV collagen IHC abnormalities in ARAS and XLAS females are the following: (i) in the typical ARAS female, renal basement membranes are entirely negative for the $\alpha_3(IV)$ and $\alpha_4(IV)$ chains, and glomerular basement membranes are completely negative for the $\alpha_5(IV)$ chain, reflecting the failure to deposit $\alpha_3\alpha_4\alpha_5(IV)$ trimers; (ii) Bowman’s capsules, distal tubular basement membranes and EBM are positive for $\alpha_5(IV)$ chains, because formation and deposition of $\alpha_5\alpha_5\alpha_6(IV)$ trimers are preserved. It is important to note that type IV collagen IHC may be normal in ARAS [26].

Direct sequencing of $COL4A5$ identifies 80–90% of males with XLAS, as well as the majority of female heterozygotes [5,27]. Unfortunately, molecular genetic analysis of $COL4A5$ is not widely available. According to the Gene Reviews website (www.genereviews.org), only one laboratory in the world offers sequencing of $COL4A5$ as a clinical test. A patient’s financial resources may determine whether or not such testing is feasible. Fortunately, the majority of XLAS patients can be accurately identified by thorough clinical examination, investigation of family history, and tissue studies [28].

Outcomes of females in animal models of XLAS

There are three animal models of XLAS. Two of these occurred spontaneously in dogs and the third was engineered in transgenic mice [29–31]. Heterozygous females in one of the canine XLAS kindred universally develop proteinuria but typically have normal renal function for many years (G. E. Lees, personal communication), limiting their usefulness for understanding phenotypic variability in XLAS females.
The persistence of normal renal function despite longstanding proteinuria in female XLAS dogs is intriguing but unexplained. Females with murine XLAS display a phenotypic range that approximates that of women with XLAS [31]. These animals should allow testing of the hypothesis that X-inactivation balance determines renal outcomes in XLAS.

Renal transplantation in women with Alport syndrome

Renal transplantation is usually very successful in women with Alport syndrome who progress to end-stage renal failure. Although anti-GBM nephritis of the renal allograft occurs in about 3% of transplanted Alport males, the risk of this complication in females with XLAS should theoretically be close to zero, since expression of even small amounts of the α5(IV) chain in their tissues should allow establishment of immunological tolerance for that protein. However, women with ARAS due to certain COL4A3 mutations can develop anti-GBM nephritis of the allograft [32].

Summary

If there is a single, ‘take-home’ message to be derived from this review, it is that Alport syndrome in women is a significant renal disorder that can result in renal insufficiency and renal failure. As with other conditions associated with chronic progressive renal disease, such as diabetes, women with Alport syndrome should be identified at an early age, subjected to careful and regular clinical monitoring and offered up-to-date prophylactic therapies once proteinuria appears.

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References

Bisphosphonates in the renal patient

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Over the past decade, bisphosphonate use in patients with various forms of kidney disease has become widespread. The extensive take-up of these agents by nephrologists reflects the twin perceptions that bisphosphonates are generally safe in patients with kidney disease, and that skeletal protection, readily demonstrable in bisphosphonate-treated populations without kidney disease, is also achievable in patients with chronic kidney disease (CKD) and other forms of nephropathy. Unfortunately, both of these perceptions are based on limited evidence and somewhat tenuous extrapolations [1,2].

The case for bisphosphonates in patients with renal disease

In the non-renal population, convincing evidence exists to show that bisphosphonates can effectively prevent, or at least attenuate, bone loss in glucocorticoid-treated patients with normal or near normal renal function and inflammatory disease [5]. It was reasonable, therefore, to hope that similar gains would be realized in bisphosphonate-treated patients with renal disease. Most studied have been patients undergoing renal transplantation, one of a cluster of scenarios associated with exceptionally rapid bone loss [9–11]. Fracture rates, already high in the dialysed CKD population, rise further following transplantation [12]. A number of small randomized studies have now been published and the consistent picture that has emerged is one of the effective protection of bone mineral density (BMD) in bisphosphonate-treated transplant recipients, generally without evidence of reduction of fracture rate [13–15]. The failure to demonstrate the clinical outcome that really matters, namely reduction of fracture rate, may reflect inadequate statistical power of the completed studies or a genuine absence of a significant impact of bisphosphonates in this patient group [16]. The latter explanation would imply that the CKD and transplanted population differ importantly from the general population, in whom evidence of clinical benefit is well established. Such a notion is certainly plausible—bone mineral density is only one of the determinants of bone strength, the others collectively falling under the umbrella of ‘bone quality’. Bone quality is a somewhat enigmatic concept that probably comprises a range of components including bone composition, micro architecture,