Aortic abnormalities in males with Alport syndrome

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membranes. The distinct networks formed by Type IV collagen are limited to a subset of renal basement membranes, including glomerular basement membranes, Bowman’s capsules, distal tubular basement membranes and various base-ments have been identified in mammalian basement mem-
bases: α1α2, α3α4α5 and α5α5α6 [2]. Networks composed of α1α1α2 trimers are found in all basement membranes. The distinct networks formed by α3α4α5 trimers are limited to a subset of renal basement membranes, including glomerular basement membranes, Bowman’s capsules, distal tubular basement membranes and certain basement membranes of the cochlea, eye and other non-renal tissues [3–6]. The α5α5α6-containing networks are also restricted in distribution and, in the kidney, have been identified in Bowman’s capsules, distal tubular basement membranes as well as in epidermal basement membranes (EBM) [5]. Recently, α1(IV), α2(IV), α5(IV) and α6(IV) chains, but not α3(IV) and α4(IV) chains, have been localized to basement membranes surrounding visceral smooth muscle cells in several organs, including the aorta [7,8].

The X-linked form of Alport syndrome (XLAS) arises from mutations in COL4A5, the gene that encodes the α5 (IV) chain [9]. In most males with XLAS, basement membranes lack the expression of α3α4α5 and α5α5α6 trimers [3,5]. Absence of α3α4α5 trimers from glomerular basement membranes results in structural and functional abnormalities of glomeruli that ultimately result in renal fibrosis. The sensorineural hearing deficit that is characteristic of Alport syndrome (AS) is a consequence of the absence of α3α4α5 trimers from cochlear basement membranes through mechanisms that have yet to be clearly defined [6].

The various roles of Type IV collagen in basement membranes are still being elucidated. The recent finding that familial porencephaly, characterized by recurrent intracerebral haemorrhage beginning in the neonatal period, is caused by mutations in COL4A1 suggested that the α1 chain of Type IV collagen [α1(IV)] performs important functions in vascular basement membranes [10–12]. Indirect evidence implicates basement membranes within microvessels penetrating the basal ganglia as defective in this disorder. More recently, Plaisier et al. have described hereditary angiopathy, nephropathy, aneurysms and muscle cramps, a disorder also arising from COL4A1 mutations in which the vascular phenotype extends to aneurysms of large arteries within the carotid system [13].

The discovery of mutations in the COL4A3, COL4A4 and COL4A5 genes in patients with AS established the significance of the Type IV collagen network formed by α3(IV), α4(IV) and α5(IV) chains for normal function of glomerular basement membranes and various basement membranes of the eye and cochlea. A role for these chains in vascular basement membrane function has not been widely suspected, although ruptured intracranial aneurysm has been described in an adolescent with AS [14], as has a ruptured thoracoabdominal aneurysm in a 36-year-old Alport male [15]. Nevertheless, the fact that a Type IV collagen network composed of α5(IV) and α6(IV) chains has been identified surrounding smooth muscle cells of murine and bovine aortas raised the possibility that this network has significant conserved functions in the aortic media [7,8].

In this report, we describe aortic abnormalities in five males with AS, ranging from asymptomatic dilation to dissection and aneurysm. We further describe the effect of targeted mutation of the COL4A5 gene on the expression of the α5(IV) chain in murine aorta. These observations suggest that the presence of a Type IV collagen network composed of α5(IV) and α6(IV) chains may play a role in maintaining vascular integrity in the aorta.

Case reports

Case 1 was diagnosed with XLAS in childhood and underwent renal transplantation at age 15. He returned to dialysis at age 25. Aortic dissection occurred 1 month after starting dialysis (Figure 1) and was treated with aortic valve replacement and aortic grafting (Table 1).

Case 2 was diagnosed with AS at age 10 and began dialysis at age 19. At age 22, he was found to have
Case 1 was diagnosed with AS by renal biopsy at age 7. He exhibited sensorineural deafness, anterior lenticonus and corneal erosions. He commenced haemodialysis at age 20 and received a transplant from his father 4 months later. At age 32, he developed a spontaneous thoracic aortic dissection (Figure 2) and underwent emergency ascending aortic graft replacement.

Immunohistochemical methods and results

Human skin biopsies

Tayel and colleagues previously described two brothers with AS and aortic disease [18] (Table 2). One of the brothers began dialysis at approximately age 10. Fatal dissection of the thoracic aorta occurred at age 13. At autopsy, the aortic valve and the diameter of the aortic root were normal. His sibling was found to have asymptomatic aortic root enlargement (4.4 cm), with a normal aortic valve, at age 15. In order to confirm the diagnosis of XLAS, this patient underwent punch skin biopsy, along with his parents, another affected male sibling and

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender</th>
<th>Alport genotype</th>
<th>Age (years) at ESKD onset</th>
<th>Age (years) at diagnosis of aortic disease</th>
<th>Aortic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>X-linked (by pedigree)</td>
<td>15</td>
<td>25</td>
<td>Thoracic aortic dissection</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>X-linked (deletion of exons 41–51 in COL4A5)</td>
<td>19</td>
<td>22</td>
<td>Aortic insufficiency, bicuspid aortic valve</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>X-linked (deletion of exons 41–51 in COL4A5)</td>
<td>18</td>
<td>21</td>
<td>Asymptomatic dilatation of ascending aorta, aortic arch, descending aorta</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>X-linked (1-bp deletion in COL4A5)</td>
<td>22</td>
<td>32</td>
<td>Ascending aortic aneurysm with rupture</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>X-linked (1-bp deletion in COL4A5)</td>
<td>20</td>
<td>32</td>
<td>Thoracic aortic dissection</td>
</tr>
</tbody>
</table>
an asymptomatic female sibling. Four-micron skin sections were stained for the \( \alpha_1(IV) \) and \( \alpha_5(IV) \) chains, as previously described [19]. As shown in Figure 3, EBM of affected males showed no staining for the \( \alpha_5(IV) \) chain. EBM of their father and asymptomatic female sibling showed strongly positive staining for \( \alpha_5(IV) \). Their mother's EBM showed a mosaic staining pattern for \( \alpha_5(IV) \), with a strongly positive region adjacent to a region in which staining for \( \alpha_5(IV) \) was absent. EBM showed strongly positive staining for \( \alpha_1(IV) \) in each family member (data not shown). These findings are diagnostic of XLAS and are consistent with the presence of a COL4A5 mutation that prevents tissue expression of the \( \alpha_5(IV) \) chain [20].

**Mouse aorta**

Sections of aorta were obtained from 3-month-old males with murine XLAS [21] and wild-type male littermates. Sections were stained with primary monoclonal antibodies to the \( \alpha_1(IV) \) or \( \alpha_5(IV) \) chain, followed by Cy5-tagged anti-mouse IgG.

Results of immunostaining are shown in Figure 4. In wild-type males, staining for \( \alpha_1(IV) \) and \( \alpha_5(IV) \) was present between elastin layers, as previously described [7]. In males with murine XLAS, staining for \( \alpha_5(IV) \) was completely absent, while staining for \( \alpha_1(IV) \) was preserved.

**Discussion**

The first report of aortic abnormalities in males with AS was published in 1991 by Tayel and colleagues who described two adolescent brothers with AS, one of whom suffered a fatal thoracic aortic dissection while the other exhibited asymptomatic aortic dissection [18] (Table 2). In the present study, we have confirmed the diagnosis of XLAS in this family by immunostaining of skin biopsy specimens. In addition, we show that a vascular phenotype is not unique to this family but is likely to be a rare complication of AS. We describe five additional males with classic AS accompanied by a vascular phenotype, including aneurysm and dissection of the thoracic aorta (Cases 1, 4 and 5), diffuse dilatation of the aorta (Case 3) and aortic insufficiency (Case 2). The most notable feature of these new cases and those previously reported is the early presentation of aortic disease. Four individuals suffered aortic dissection at 13, 25, 32 and 32 years of age, respectively; in the general population, aortic dissection occurring before age 40 is unusual [22]. Although abdominal aortic aneurysm is typically a disease of the elderly with a mean age at diagnosis of 72 years [23], a previously reported Alport male with abdominal aortic aneurysm was 36 years of age at diagnosis [15] (Table 2). A report describing a 14-year-old male with AS who experienced rupture of an intracranial aneurysm suggests that, if there is truly a vascular phenotype of AS, it may extend beyond the aorta [14]. Case 4 in the present series exhibited aneurysms of the brachial, axillary and subclavian arteries, possibly related to arteriovenous fistula created for dialysis access.

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**Table 2. Patient characteristics (previously published)**

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Gender</th>
<th>Alport genotype</th>
<th>Age (years) at ESKD onset</th>
<th>Age (years) at diagnosis of aortic disease</th>
<th>Aortic findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Male</td>
<td>X-linked</td>
<td>15</td>
<td>15</td>
<td>Asymptomatic aortic root enlargement</td>
</tr>
<tr>
<td>18</td>
<td>Male</td>
<td>X-linked</td>
<td>10</td>
<td>13</td>
<td>Thoracic aortic dissection</td>
</tr>
<tr>
<td>15</td>
<td>Male</td>
<td>X-linked</td>
<td>16</td>
<td>36</td>
<td>Abdominal aortic aneurysm with rupture</td>
</tr>
</tbody>
</table>
Fig. 3. Pedigree and results of skin biopsy immunostaining for α5(IV) in members of the family described by Tayel et al. [18]. The father was unaffected and exhibited continuous staining of EBM for α5(IV). Two of four affected sons underwent skin biopsies and showed absence of EBM staining for α5(IV), while normal staining was observed in an unaffected daughter. The mother was heterozygous for XLAS and displayed mosaic staining of EBV for α5(IV). Empty symbols indicate unaffected family members, filled boxes indicate affected males and the cross-hatched symbol indicates a heterozygous female. A colour version of this figure is available online.

Fig. 4. Results of immunostaining of aortic sections from wild-type mice and mice with XLAS due to a nonsense mutation in the col4a5 gene. In both wild-type and Alport mice, staining for α1(IV) is observed between autofluorescent elastic laminae. Similar localization of staining for α5(IV) is observed in wild-type mice, but staining for α5(IV) is negative in Alport mice. It is not clear whether the apparent contraction of the Alport aortic segment is related to the Type IV collagen abnormality or represents an artefact of tissue preparation. A colour version of this figure is available online.
Aortic abnormalities in males with Alport syndrome

The early onset of vascular disease in these males with AS strongly suggests that the lack of an intact α5β5γ6(IV) network in the aortic media is a contributing pathogenic factor. Although we were unable to directly assess α5(IV) protein expression in aortic media in these patients, several lines of evidence suggest that α5(IV) expression is likely defective if not entirely absent. First, we show in this report that, in male mice with a nonsense COL4A5 mutation [21], the aortic media exhibits no staining for α5(IV). Second, COL4A5 mutations in Case 3 (partial deletion) and Case 4 (nonsense) would have prevented α5(IV) protein expression. Third, absence of α5(IV) protein expression in EBM was demonstrated in a patient previously reported by Tayel and colleagues [18] and, by extension, in his sibling who had suffered fatal thoracic aortic dissection. In the remaining cases (1, 2 and 5), the onset of terminal renal failure before age 25 suggests the presence of COL4A5 mutations that would prevent or severely diminish α5(IV) protein expression [24]. Although terminal renal failure before age 25 also occurs frequently in patients with autosomal recessive AS, these individuals should have unaltered expression of the α5α5α6(IV) network in aortic media, as they do in Bowman’s capsules, tubular basement membranes and EBM [9].

The lamellar unit, consisting of two elastic lamellae and the smooth muscle cells and extracellular matrix (ECM) material between them, is the basic structural element of the aortic media [25]. The ECM of the lamellar unit contains several collagen types, including Type IV collagen, as well as fibrillins, fibronectin, proteoglycans and other proteins [26]. Histological studies of aortic dissections have shown defective expression of Type IV collagen locally around medial smooth muscle cells [27].

The link between AS and a vascular phenotype is consistent with an established association between aortic wall abnormalities and mutations in other ECM proteins [28]. The best characterized of these disorders is Marfan syndrome, which is notable for early onset aortic root aneurysm/dissection as well as diffuse skeletal and ocular manifestations [29]. The Marfan syndrome is caused by mutations in the fibrillin-1 gene, which encodes the principal component of ECM microfibrils [29]. Until recently, it was assumed that this pathogenic mechanism underlying the Marfan phenotype had to do with a structural failure of the ECM. However, recent work suggests that increased activity of the TGF-β signalling pathway may play a critical role in disease pathogenesis [29]. Although mutations in COL4A5 could result in a simple structural flaw of the aortic wall, we cannot exclude aberrant signalling that could also ensue from defects in the ECM surrounding vascular smooth muscle cells.

Interestingly, one of the siblings described by Tayel et al. [18] had features reminiscent of Marfan syndrome. His primary vascular finding was asymptomatic enlargement of the aortic root, but he was also noted to be tall and slender with dolichostenomelia (long, thin extremities), maxillary hypoplasia, prominence of the mandible, pectus carinatum and scoliosis. He did not have arachnodactyly or ectopia lentis. His sibling, who had fatal dissecting aortic aneurysm, did not display Marfan-like features [18]. It is tempting to speculate that fibrillin variants act as modifiers of AS or vice versa.

Although the cases we describe here are dramatic, most males with XLAS do not exhibit severe vascular complications. We also note that, in a murine model of XLAS, male mice do not develop overt aortic dissections, despite absent aortic staining for α5(IV) collagen. There are several possible explanations for these phenotypic differences. Aortic abnormalities associated with COL4A5 mutations could be the result of a predisposition to aortic wall weakness in combination with environmental stressors such as poorly controlled hypertension or a bicuspid aortic valve, which has been linked with aortic root dilatation [22]. Systemic hypertension secondary to chronic kidney disease has also been described as a risk factor for aortic dissection in young patients [30]. An interaction between Type IV collagen mutations and environmental stress has been invoked to explain haemorrhagic stroke in a subset of patients with mutations in the COL4A1 gene [10].

Our findings suggest that vascular abnormalities such as aortic dilatation, aortic aneurysm and aortic dissection are features of AS. The frequency of these aortic abnormalities has not been systematically studied in a cohort of Alport patients and deserves further evaluation. It may be prudent to include aortic imaging in the evaluation of Alport patients who are approaching terminal renal failure, especially if they have a family history of vascular abnormalities, with periodic reassessment following transplantation.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

References

2. Bouthaud A, Borza DB, Bondar O et al. Type IV collagen of the glomerular basement membrane: evidence that the chain specificity of
Selection on albuminuria enhances the efficacy of screening for cardiovascular risk factors

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