

## **Expert Guidelines for the management of Alport syndrome and TBMN**

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**Abbreviations:** GBM - glomerular basement membrane, RBC - red blood cells, TBMN – Thin basement membrane nephropathy

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## **Abstract**

There are few prospective, randomised controlled clinical trials that address the diagnosis and management of patients with Alport syndrome or Thin basement membrane nephropathy. The following Recommendations have been developed by adult and paediatric Nephrologists, and Geneticists from four continents whose clinical practice focuses on these conditions. The 18 Recommendations are based on Level D ('Expert opinion without explicit critical appraisal, or based on physiology, bench research or first principles', National Health Service category) or Level III evidence ('Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees, US Preventive Services Task Force).

The Recommendations include the use of genetic testing as the 'gold standard' for the diagnosis of Alport syndrome and the demonstration of its mode of inheritance; the need to identify and follow all affected members of a family with X-linked Alport syndrome, including the mothers of affected males; the treatment of males with X-linked Alport syndrome and individuals with autosomal recessive disease with angiotensin converting enzyme (ACE) inhibitors, possibly even before the onset of proteinuria; discouraging the affected mothers of males with X-linked Alport syndrome from renal donation because of their own risk of kidney failure; and consideration of genetic testing to exclude X-linked Alport syndrome in some individuals with Thin basement membrane nephropathy. The authors recognise that as further evidence, including data from Patient Registries, emerges, these Guidelines will continue to evolve.

## Introduction

Persistent glomerular hematuria present for at least a year occurs in at least 1% of the population<sup>1-3</sup> and is typically due to Thin basement membrane nephropathy (TBMN), and, much less often, to Alport syndrome.<sup>3-5</sup> However recognition of Alport syndrome is more important because of its inevitable progression to end-stage renal failure and the ability of treatment to slow down the rate of progression.

Alport syndrome is characterised by hematuria, progressive renal failure, hearing loss, lenticonus, and retinal flecks,<sup>6</sup> a lamellated glomerular basement membrane (GBM)<sup>6</sup> with an abnormal collagen IV composition,<sup>7</sup> and mutations in the *COL4A5* or *COL4A3/COL4A4* genes.<sup>8,9</sup> Eighty-five per cent of families have X-linked inheritance with mutations in the *COL4A5* gene<sup>10</sup> and most of the others have autosomal recessive disease with homozygous mutations or compound heterozygous mutations in both copies (*in trans*) of either the *COL4A3* or *COL4A4* gene.<sup>9</sup> Autosomal dominant inheritance is very rare, and results from heterozygous *COL4A3* or *COL4A4* variants.<sup>11</sup>

Individuals with TBMN have isolated haematuria, and normal renal function, hearing and ocular examination.<sup>3</sup> TBMN is usually caused by heterozygous *COL4A3* or *COL4A4* mutations, and often represents the carrier state of autosomal recessive Alport syndrome.<sup>3</sup>

Alport syndrome and TBMN may be clinically and ultrastructurally indistinguishable, and some clinicians mistakenly use the term 'TBMN' in women and boys with X-linked Alport disease. The distinction between these conditions is, however, critical because of the different risks of renal failure and other complications for the individual and their family members.

This document provides: definitions for Alport syndrome and TBMN; a diagnostic algorithm for the patient with persistent hematuria; a description of the clinical features in Alport syndrome and how they contribute to the certainty of the diagnosis; a list of other diseases that share clinical features with Alport syndrome; and criteria that help distinguish between X-linked and autosomal recessive inheritance.

The Recommendations provided here discuss the use of the terms Alport syndrome and TBMN (Recommendation 1), criteria for the diagnosis of Alport syndrome (2), the distinction between X-linked and autosomal recessive inheritance (3), predicting the clinical phenotype from the *COL4A5* mutation (4), the importance of identifying other affected family members (5), genetic counselling (6), ongoing medical management (7), peri-transplantation care (8), the affected female – diagnosis, management and renal donation (9), and for autosomal recessive Alport syndrome: family screening (10); genetic counselling (11); management (12), and renal donation (13). The Recommendations for TBMN include the criteria for diagnosis (14), genetic testing (15), management and poor prognostic indicators (16), family screening (17), and renal donation (18).

Prospective randomised controlled clinical trials for the diagnosis and management of Alport syndrome and TBMN are difficult to undertake because of the small number of patients at any individual treatment centre, and the patients having different stages of disease. Instead these Recommendations address the major issues in Alport syndrome and TBMN and are based on the experience and opinion of the authors, as well as retrospective studies in humans, animal experiments, and data from Alport Registries.

Nevertheless the authors were able to reach consensus on all the Recommendations and considered the Recommendations all outweighed any associated risk. The authors were guarded only in suggesting the time to introduce renin-angiotensin blockade in X-linked Alport syndrome before the studies were completed. Where there is scientific evidence for any Recommendation this is indicated in the preamble of each section.

## DEFINITIONS

The distinction between Alport syndrome and TBMN is critical but may be difficult in females and boys with X-linked disease who have hematuria and GBM thinning, but not the characteristic Alport hearing loss, lenticonus or retinopathy. The term TBMN should not be used in females or boys with X-linked Alport syndrome even when they have GBM thinning. The GBM usually also has stretches of splitting or lamellation in this or a subsequent renal biopsy, or in a biopsy from another affected family member. Further clinical or genetic testing may be required. Clinicians should understand that some patients with TBMN develop renal failure because of coincidental glomerular or other renal disease.<sup>12</sup>

**Recommendation 1.** The term Alport syndrome should be reserved for patients with the characteristic clinical features, a lamellated GBM with an abnormal collagen IV composition, and where a *COL4A5* mutation (X-linked disease), or two *COL4A3* or two *COL4A4* mutations (*in trans* or on different chromosomes, consistent with autosomal recessive disease) are identified or expected.

The term Thin basement membrane nephropathy (TBMN) should be reserved for individuals with persistent isolated glomerular hematuria who have a thinned GBM due to a heterozygous *COL4A3* or *COL4A4* (but not *COL4A5*) mutation. TBMN should not be used where there is a thinned GBM and the diagnosis is likely to be X-linked Alport syndrome. This distinction is to ensure patients who have X-linked Alport syndrome are not falsely reassured by TBMN's usually benign prognosis.

Alport syndrome should not necessarily be diagnosed where there is renal impairment together with a heterozygous *COL4A3* or *COL4A4* mutation. This is more likely to be due to TBMN on population frequencies together with a coincidental renal disease (such as IgA glomerulonephritis) or to autosomal recessive Alport syndrome with a second, undetected mutation. In these circumstances, the correct diagnosis may require further discussions between the Nephrologist, Pathologist, Clinical Geneticist, Ophthalmologist, and Audiologist and interpretation of the relevant tests.

## **DIAGNOSIS OF ALPORT SYNDROME**

Alport syndrome is suspected when there is persistent glomerular hematuria. The likelihood increases if there is a family history of Alport syndrome and no other cause for the hematuria, or the characteristic clinical features (hearing loss, lenticonus or retinopathy) are present, or the GBM lacks the collagen IV  $\alpha 3$  and  $\alpha 5$  chains (**Figure 1**). The diagnosis is confirmed if there is a lamellated GBM or a pathogenic mutation in the *COL4A5* gene or two pathogenic mutations in either the *COL4A3* or the *COL4A4* gene. The sensitivity and specificity of each feature is described for X-linked Alport syndrome in **Table 1**.<sup>13</sup> Genetic testing is at least 90% sensitive for X-linked disease.<sup>14</sup>

Alport syndrome must be distinguished from the other causes of inherited hematuria; hematuria and hearing loss; retinal flecks; and a lamellated GBM (**Table 2**). Hematuria is

not typical of the commonest familial forms of pediatric renal failure, namely, focal segmental glomerulosclerosis and nephronophthisis. Hearing loss is common in different forms of inherited renal disease but also occurs coincidentally with middle ear infections, increasing age, and industrial noise exposure. Other causes of GBM lamellation are very rare or have further distinctive features.

**Recommendation 2. The diagnosis of Alport syndrome is suspected when an individual has glomerular hematuria or renal failure, and a family history of Alport syndrome or renal failure, without another obvious cause. These individuals should undergo testing for microalbuminuria/proteinuria, as well as audiometry, formal ophthalmological examination, and, preferably, renal biopsy for GBM ultrastructure, collagen IV composition (if available), and an assessment of damage.**

**The diagnosis of Alport syndrome is highly likely if there is glomerular hematuria and a family history of Alport syndrome with no other cause for the hematuria; or if bilateral high tone sensorineural hearing loss, lenticonus or fleck retinopathy is present; or if the GBM lacks the collagen IV  $\alpha$ 5 chain. The diagnosis of Alport syndrome is confirmed with the demonstration of a lamellated GBM, or a *COL4A5*, or two *COL4A3* or *COL4A4* mutations.**

**In individuals where the diagnosis is still unclear, and genetic testing is not available, it is often useful to examine the child's mother or an older affected male relative using the tests and algorithm described.**

## **MODES OF INHERITANCE**

Once the diagnosis of Alport syndrome has been made, it is important to distinguish between X-linked and autosomal recessive inheritance because of the different implications, including the risk of renal failure, for family members.

X-linked Alport syndrome is five times more common than recessive disease.<sup>10</sup> The mode of inheritance is sometimes suspected from the pedigree (X-linked disease: 'skipping a generation' where there is an affected female with hematuria but no other features; autosomal recessive disease: disease in a single generation, males and females affected equally often and equally severely, and a father with hematuria) or gender of the affected

individual (recessive disease: female with renal failure, hearing loss, lenticonus or retinopathy). Inheritance is usually confirmed with genetic testing. Sometimes the GBM collagen IV composition is used but this is only 80% sensitive at best, testing is not widely available and interpretation may be difficult especially in females with X-linked disease.<sup>15</sup> Features that distinguish between X-linked and autosomal recessive Alport syndrome are summarised in **Table 3**.

**Recommendation 3. The mode of inheritance of Alport syndrome is determined most accurately with the demonstration of a pathogenic mutation in the COL4A5 gene, or two mutations in either the COL4A3 or COL4A4 gene on different chromosomes.**

### **X-LINKED ALPORT SYNDROME**

Most patients with X-linked Alport syndrome have another family member with hematuria, since only 15% mutations occur *de novo* and penetrance is 95%.<sup>16</sup> Other X-linked causes of hematuria and renal failure are very uncommon.

Males with X-linked Alport syndrome who develop end-stage renal failure before the age of 30 years usually have extra-renal manifestations,<sup>13,17</sup> but those with late onset renal failure may have only hearing loss **Table 3**). The high tone sensorineural hearing loss occurs in 70%, and lenticonus in up to 30% of affected males by the fourth decade, when renal failure, hearing loss and the retinopathy are already present. The central fleck (50%) and peripheral coalescing (60%) retinopathies are common. Females have variable clinical features depending on X chromosome inactivation in individual tissues, and their features are described separately.

GBM lamellation is usually extensive in adult males. The GBM is initially thinned in boys with focal lamellation<sup>18</sup> that becomes more extensive with age. The GBM collagen IV composition is often abnormal.<sup>7,19</sup> The GBM typically lacks the  $\alpha3\alpha4\alpha5$  network, and the epidermal membrane has no  $\alpha5$  chain.<sup>20</sup> Examination of a skin biopsy is less invasive and just as fast as a renal biopsy.<sup>20</sup>

### **Genetic testing**

Genetic testing is useful when Alport syndrome is suspected but cannot be confirmed with other techniques, and when TBMN is suspected but X-linked Alport syndrome must be excluded (**Table 4**). Most ethical concerns related to testing minors for Alport syndrome are outweighed by the potential of treatment to delay end-stage renal failure.<sup>21</sup> Further information on molecular testing for Alport syndrome is available at [www.genereviews.org](http://www.genereviews.org) (**Table 5**).

The mutation detection rate in X-linked Alport syndrome is optimal using a combined approach of sequencing genomic DNA, hair root or skin cDNA followed by multiplex ligation-dependent probe amplification (MLPA) to detect large deletions, insertions or duplications.<sup>22-26</sup> Current techniques identify mainly coding region variants. Mutations are more likely to be identified in individuals with early onset renal failure and extrarenal features,<sup>13,17,27</sup> because the diagnosis of Alport syndrome is more likely to be correct.

The mutations are different in each family with X-linked Alport syndrome and more than 700 variants have been described ([http://grenada.lumc.nl/LOVD2/COL4A/home.php?select\\_db=COL4A5](http://grenada.lumc.nl/LOVD2/COL4A/home.php?select_db=COL4A5)).<sup>14</sup> Clinical features depend mainly on the mutation's location and nature. About 50% result in a stop codon either directly or downstream, and 40% of mutations are missense.<sup>14</sup> Large deletions and rearrangements, nonsense mutations, and carboxy terminal missense mutations typically result in early onset renal failure, hearing loss, and ocular abnormalities,<sup>13,27</sup> whereas amino terminal missense mutations are often associated with late onset renal failure without the extra-renal features. The likelihood of early onset renal failure can also be predicted from the effect of the mutation in other family members.<sup>27</sup>

Genetic linkage studies are used rarely to exclude a mode of inheritance in families where no mutation has been demonstrated,<sup>15</sup> and, sometimes, in prenatal or preimplantation genetic diagnosis where the mutation is not known.<sup>28</sup>

Individuals with suspected Alport syndrome but no *COL4A5* mutation may have a deletion, splice site or a deep intronic variant in *COL4A5*, autosomal recessive Alport syndrome, or another inherited nephropathy.



**Recommendation 4. The demonstration of a pathogenic *COL4A5* variant confirms the diagnosis of Alport syndrome and X-linked inheritance. The mutation's location and nature may help predict the likelihood of early-onset renal failure and extrarenal features. These are sometimes already obvious from examination of other affected family members. The mutation itself or a disease-associated haplotype can be used in preimplantation and prenatal diagnosis.**

#### **Screening members of a family with X-linked Alport syndrome**

All affected members of a family with X-linked Alport syndrome, including females, should be identified because of their own risk, and their offspring's risk, of renal failure. Each of the sons of a female with X-linked Alport syndrome has a 50% risk that he is affected and will develop renal failure, and each of her daughters has a 50% risk of being affected too. However, while a male with X-linked disease can be reassured that none of his sons will inherit the mutation, all of his daughters, and half of her sons and daughters, will be affected too. Thus the risks are greater for the offspring of the female with X-linked disease than the affected male.

In any family with X-linked Alport syndrome, individuals with hematuria are highly likely to be affected too, but other coincidental hematuria must be excluded. When the mutation in any family is known, genetic testing can be used to confirm the affected status.

**Recommendation 5. All affected members of a family with X-linked Alport syndrome, including females, should be identified. Thus most mothers of affected boys are also affected. At-risk family members should be screened for hematuria on at least 2 occasions, and offered other screening tests, but genetic testing is preferred especially if a mutation has already been identified in the family ('cascade testing').**

#### **Genetic counselling**

**Recommendation 6. Affected individuals should be referred to an interested Nephrologist for long term management, and offered a consultation with a Clinical Geneticist to discuss the disease, its inheritance, and the indications for genetic testing of other family members. There should be a non-directive discussion about**

**available reproductive options, including prenatal and preimplantation genetic diagnosis, preferably prior to any pregnancy.**

**Individuals and their families should be advised of their diagnosis, their risk of renal failure, and their children's likelihood of inheriting the causative mutation and thus developing renal failure. Affected individuals should be advised of the availability of local, national and international Patient Support Groups and relevant websites (Table 5). They should also be encouraged to participate in patient Registries that will help improve understanding of Alport syndrome and its management.**

### **Monitoring and treatment**

Proteinuria, hearing loss, lenticonus, retinopathy, and reduced levels of GBM collagen IV  $\alpha$ 5 chain all correlate with an increased likelihood of early onset renal failure in males<sup>16,28,29</sup> but the risks have not been studied prospectively. Hearing continues to deteriorate in adulthood and is helped with hearing aids, but affected individuals should protect their hearing from additional insults throughout life. The lenticonus also worsens but can be corrected with lens replacement.<sup>30</sup> The retinopathy progresses but does not affect vision nor require treatment.

Angiotensin converting enzyme (ACE) inhibitors reduce proteinuria in children with X-linked Alport syndrome.<sup>31</sup> Angiotensin receptor blockers and aldosterone inhibitors have additional benefits on proteinuria.<sup>32</sup> Evidence from a single retrospective study, animal models and other forms of renal failure suggest that ACE inhibitors delay the onset of end-stage renal failure and improve life-expectancy in adult males, even when commenced prior to the onset of proteinuria.<sup>33</sup> **However it is critical that the effect of renin-angiotensin blockade on proteinuria and renal failure progression is formally evaluated and this is the aim of the EARLY PRO-TECT study (EU Clinical Trials Register). One approach in the mean time is to target those individuals at greatest risk of early-onset renal failure<sup>34</sup>.**

Other potential therapies include statins,<sup>35</sup> metalloproteinase inhibitors,<sup>36</sup> vasopeptidase inhibitors,<sup>37</sup> chemokine receptor antagonists,<sup>38</sup> bone marrow transplantation and stem cell therapy.<sup>39,40</sup>

**Recommendation 7. Males with X-linked Alport syndrome should be managed lifelong by a Nephrologist and have their risk factors for progressive renal failure optimised including careful management of their hypertension, proteinuria, and dyslipidemia. Treatment with ACE inhibitors, even before the onset of proteinuria, especially in individuals with genetic mutations or a family history consistent with early onset renal failure, may delay the onset of end-stage disease and improve life expectancy.**

**Affected individuals should avoid ototoxic medication and industrial noise exposure to minimise further hearing loss.**

### **Renal transplantation**

Patients with X-linked Alport syndrome who undergo transplantation have similar or better survival rates and graft survival rates than in other inherited renal diseases,<sup>41</sup> except possibly where the kidney is from an affected female donor.<sup>42</sup> **Affected female family members should be strongly discouraged from donating a kidney, but where this has occurred, the recipient should receive nephroprotective treatment, such as renin-angiotensin system blockade, from the time of surgery.**

Three - 5 % of males develop antiGBM disease with rapid allograft loss after transplantation.<sup>29,43,44</sup> AntiGBM disease is more common with large gene deletions,<sup>45</sup> but occurs also with other mutations.<sup>29</sup> In these individuals, the risk of antiGBM disease is higher after subsequent renal transplants, and circulating antiGBM antibodies **are best demonstrated with GBM immunohistochemistry and less effectively with an antiGBM ELISA because of different epitope specificities.**<sup>46</sup>

**Recommendation 8. Males with X-linked Alport syndrome and increased risk of antiGBM disease post-transplant (early onset renal failure, extrarenal features) should be monitored closely and undergo prompt allograft biopsy for new onset glomerular hematuria, proteinuria or elevated serum creatinine.**

### **X-linked Alport syndrome in females**

Almost all females with X-linked Alport syndrome (95%) have hematuria and many eventually develop other clinical features, especially proteinuria (75%),<sup>16</sup> end-stage renal failure (15% by the age of 60), hearing loss (40%) or peripheral retinopathy (40%).<sup>17,47</sup> Lenticonus may not occur in females at all, and the central retinopathy is rare. It is, thus, debatable whether females should be considered 'affected' or 'carriers'. Those of us who prefer the term 'affected' maintain it conveys the risks for any female and the need for ongoing monitoring and treatment.

Most (85%) mothers of affected boys also have the mutation, but many are asymptomatic, and 80% are only diagnosed after their son or another male relative has presented.

The GBM in affected females is typically thinned with focal areas of lamellation that become more extensive with time.<sup>48</sup> The collagen IV  $\alpha3\alpha4\alpha5$  network is patchily present depending on X chromosome inactivation and resulting in the distinctive immunohistochemical findings.

Renin-angiotensin system antagonists are nephroprotective in females with X-linked Alport syndrome and should be used to treat those with hypertension, proteinuria and other risk factors for renal failure progression.<sup>49</sup> Again there is preliminary support, **but still no evidence**, for a benefit from treatment with ACE inhibitors even before the onset of proteinuria.<sup>50</sup> Poor prognostic markers in females include episodes of macroscopic hematuria in childhood and proteinuria.<sup>42,51,52</sup> A renal biopsy is warranted if there is significant proteinuria (> 1 g/day) or renal impairment. However **changes in the renal biopsy and GBM may be patchy, sampling variation is common, and interpretation may be difficult.**<sup>53</sup> Sometimes females with X-linked Alport syndrome themselves require a transplant for renal failure, but they do not develop antiGBM disease post-transplant.<sup>28</sup>

A female family member commonly considers donating one of her kidneys to an affected son or brother. The low *de novo* mutation rate means that most mothers (85%) of affected males also have the mutation. A sister's risk of having the mutation is about 50% if her mother is also a carrier. Carrier family members who proceed with donation have an increased risk of renal failure in later life although the extent of this increase is not known. Affected donors also have an increased risk of hypertension and microalbuminuria/proteinuria compared with other donors.<sup>54</sup> A kidney biopsy is mandatory

in a mutation-carrying potential donor, even those with normal renal function and normal levels of proteinuria, to assess renal damage resulting from the effects of random X-inactivation.<sup>42</sup> Female carriers should be kidney donors 'of last resort'.<sup>55</sup>

Conversely, 15% of the mothers of affected boys are not carriers and may donate a kidney to their son without an increased risk of renal failure. They should still undergo renal biopsy to assess damage and genetic testing to exclude X-linked Alport syndrome.

The risk of pre-eclampsia is increased in affected females with proteinuria, hypertension or renal impairment, and pregnancy may accelerate any decline in renal function already present.<sup>56</sup> Pre-existing hypertension and renal impairment predict an increased risk of obstetric complications<sup>57</sup> and proteinuria, hypertension and renal impairment are all associated with preterm delivery.<sup>57</sup>

**Recommendation 9: Females carriers of X-linked Alport syndrome typically have a good renal outcome but 15% develop end-stage renal failure by the age of 60 years. Thus the 'carrier' state should be viewed as an 'at-risk', rather than a benign, condition. Clinicians should endeavour to accurately convey this information in a way that encourages regular follow-up examinations for signs of progression, such as the development of microalbuminuria, proteinuria or hypertension, and for hearing loss, without engendering undue anxiety.**

**Some women with hematuria may want the diagnosis of Alport syndrome confirmed or excluded, prior to making reproductive decisions. This requires genetic testing.**

**Most mothers of an affected boy are carriers and may be clinically affected. Clinicians caring for an affected child should explain to the mother the importance of ascertaining her status, and refer her to a Clinical Geneticist for predictive testing if the mutation is known in the family and to a Nephrologist for clinical assessment and management. Assessment includes a renal biopsy if proteinuria or renal impairment is present.**

**Carrier females should be monitored carefully, and treated with renin-angiotensin-aldosterone blockade, if they develop hypertension, microalbuminuria or renal impairment.**

**Carrier females should be strongly discouraged from kidney donation because of their own increased risk of renal impairment and hypertension. A kidney biopsy is mandatory pre-donation to accurately determine the extent of renal damage and further discourage donation if the damage is severe. If a female carrier proceeds with donation, she must be aware of the risks of developing renal failure in later life, and should use nephroprotective strategies to minimise the effects of hypertension and proteinuria from the time of surgery.**

**Fifteen % of boys with X-linked Alport syndrome are affected as the result of a spontaneous gene mutation and their mothers are not carriers. These women should have disease excluded by testing for hematuria, and, preferably, by genetic testing.**

### **AUTOSOMAL RECESSIVE ALPORT SYNDROME**

Clinical features in autosomal recessive Alport syndrome are the same as for males with X-linked Alport syndrome. Autosomal recessive inheritance is suspected where disease is sporadic, occurs in a single generation or a consanguineous family, where males and females in a family are affected equally often and severely, where the father also has hematuria, or where a female has renal failure, lenticonus, or a central retinopathy. Autosomal recessive inheritance is confirmed when there are two *COL4A3* or two *COL4A4* pathogenic mutations or the GBM lacks the collagen IV  $\alpha3$ ,  $\alpha4$  and  $\alpha5$  chains but the  $\alpha5$  chain persists in Bowman's capsule, and the distal tubular and the epidermal membrane.<sup>19,58</sup>

#### **Genetic testing**

Genetic testing is useful to confirm the diagnosis of autosomal recessive Alport syndrome<sup>14</sup> where it is suspected on the basis of clinical features, family history or renal immunohistochemistry. Fewer mutations have been described for recessive than for X-linked disease, and too few are known for genotype-phenotype correlations. Usually both the *COL4A3* and *COL4A4* genes are examined. Two mutations will be present in one of

these genes, and, where possible, the laboratory should confirm that they affect different chromosomes by testing both parents of the affected individual. Sometimes only one mutation is identified and the other is presumed present but undetectable, consistent with autosomal recessive, rather than the very rare, autosomal dominant, inheritance.

### **Screening for affected family members**

**Recommendation 10: Parents, siblings and offspring of the individual with autosomal recessive Alport syndrome should be tested for hematuria, proteinuria and renal impairment, and preferably undergo cascade testing for the causative mutations. Those with a heterozygous mutation should be managed as for TBMN.**

### **Genetic counselling**

Individuals with autosomal recessive Alport syndrome are typically from a single generation within a family, and males and females are affected with equal frequency and severity. The situation is more complicated where the family has multiple examples of consanguinity. The risk of a sib of an individual with autosomal recessive Alport syndrome also being affected is, on average, one in four. In general, each parent of an individual with autosomal recessive Alport syndrome is an 'obligate carrier' and will be heterozygous for one of the causative mutations. Likewise, each offspring of an individual with autosomal recessive disease will also inherit one of the causative mutations. The parents and offspring have the same phenotype as TBMN with a low risk of renal failure.

**Recommendation 11. Individuals with autosomal recessive Alport syndrome should be referred to an interested Nephrologist for long term management, and offered the opportunity to consult a Clinical Geneticist to discuss the disease, its inheritance pattern and the risks for other family members. A non-directive discussion about the reproductive options, including prenatal and preimplantation genetic diagnosis, should take place preferably prior to any pregnancy.**

**Individuals and their families should be advised of their diagnosis, risk of renal failure, and their children's risk of inheriting one or more of the mutations and developing renal failure. Affected individuals should be advised of the availability of local, national and international Patient Support Groups and relevant websites.**

**They should also be encouraged to participate in Registries to help improve understanding of Alport syndrome and its management.**

### **Monitoring and treatment**

Evidence from a small retrospective Registry analysis suggests that renin-angiotensin-aldosterone system blockade, for example with ACE inhibitors, delays renal failure and improves life expectancy in individuals with autosomal recessive Alport syndrome and also improves the outlook in carriers.<sup>33,49</sup>

**Recommendation 12: Individuals with autosomal recessive Alport syndrome should be managed lifelong by a Nephrologist and have their risk factors for progressive renal failure optimised including careful management of their hypertension, proteinuria, and dyslipidemia. Again treatment with ACE inhibitors, from the time of diagnosis, even before the onset of proteinuria, may delay the onset of renal failure and improve life expectancy. Affected individuals should avoid ototoxic medication and industrial noise exposure to minimise further hearing loss.**

### **Renal donation**

Individuals with only one of the mutations that contribute to autosomal recessive Alport syndrome (for example, the parent, offspring or **some sibs** of an affected individual) have a phenotype identical to that of TBMN. They can usually be kidney donors if a pre-donation renal biopsy excludes significant renal damage and genetic testing excludes X-linked Alport syndrome.<sup>3,59</sup>

**Recommendation 13: Individuals from families with autosomal recessive Alport syndrome who have only one of the causative mutations (parents, offspring, sibs) may be renal donors if they have normal microalbuminuria, blood pressure, and renal function, and if coincidental renal disease has been excluded by renal biopsy, and X-linked Alport syndrome has been excluded by genetic testing.**

## **THIN BASEMENT MEMBRANE NEPHROPATHY (TBMN)**

### **Definition**

TBMN affects 1% of the normal population, and is characterised by haematuria, proteinuria  $\leq 200$  mg/L, normal blood pressure, normal renal function and a thinned GBM



**(Table 6)**<sup>3</sup>. TBMN usually represents the carrier state for autosomal recessive Alport syndrome, and inheritance is autosomal dominant. The prognosis is usually good, but there is an increased risk of proteinuria, hypertension, and renal impairment.<sup>3</sup> The risk of renal failure is also increased if there is coincidental renal disease or diabetes.<sup>3</sup> However it remains important to exclude X-linked Alport syndrome in these patients.<sup>3</sup>

### **Diagnosis**

TBMN is usually suspected clinically, and a renal biopsy is required only where features are atypical. The most commonly-used method for the diagnosis of TBMN is the demonstration of a thinned GBM with a width less than 250 nm or a measurement specific to a laboratory and adjusted for age and gender,<sup>60</sup> and thinning that involves at least 50% of the GBM, without the lamellation or immunohistochemical features found in Alport syndrome. However, the lamellation may be patchy and occasionally diagnostic errors are made using the GBM appearance, especially in boys and in females.

Heterozygous *COL4A3* and *COL4A4* mutations also cause autosomal dominant Alport syndrome.<sup>61</sup> The features that distinguish between mutations that cause TBMN or autosomal dominant Alport syndrome are not known. The diagnosis of autosomal dominant Alport syndrome is reserved for individuals with a lamellated GBM and autosomal dominant inheritance. Some reports of so-called autosomal dominant Alport syndrome are likely to represent TBMN with a coincidental glomerular or tubulointerstitial disease, such as IgA glomerulonephritis.<sup>12</sup> Misdiagnosis when the diagnosis is actually TBMN means that family members will be misinformed about their likelihood of renal failure.

**Recommendation 14. TBMN is usually suspected clinically where there is persistent glomerular hematuria, no proteinuria, and normal blood pressure and renal function, without another obvious explanation. There may be a family history of hematuria, but not of Alport syndrome or renal failure (except in families with autosomal recessive Alport syndrome).**

**Individuals with suspected TBMN should undergo renal biopsy if they have atypical features (proteinuria > 0.5 g/day, renal impairment) eGFR < 90 ml/min), or if X-**

**linked Alport syndrome or a coincidental glomerular or tubulointerstitial abnormality cannot be excluded.**

### **Genetic testing**

TBMN is caused by a heterozygous mutation in the *COL4A3* or *COL4A4* gene. Mutations are typically different in each family, and testing of both the *COL4A3* and *COL4A4* genes is usually required. This is labour-intensive and expensive, and it is usually more important, in an individual with only hematuria, to exclude a *COL4A5* mutation and hence X-linked Alport syndrome, rather than to make a positive molecular diagnosis of TBMN.

**Recommendation 15. Genetic testing for *COL4A3* and *COL4A4* mutations is not usually required for the diagnosis of TBMN. Screening for *COL4A5* mutations is often more important to exclude X-linked Alport syndrome.**

### **Monitoring and treatment**

The prognosis of TBMN is usually good.<sup>3</sup> However some individuals develop hypertension, proteinuria or renal impairment, which are all risk factors for progression to end-stage renal failure. Again, there is preliminary support from a single retrospective study in humans, a murine model of TBMN, and experience in other forms of diabetic and non-diabetic renal disease, that renin-angiotensin blockade delays progression to end-stage renal failure in at-risk individuals.<sup>49, 62-64</sup>

**Recommendation 16. Individuals diagnosed with TBMN should be assessed at presentation for poor prognostic indicators (hypertension, proteinuria, renal impairment). Those with poor prognostic indicators should be managed by a renal physician, and treatment should include an ACE inhibitor to delay the onset of renal failure. Other individuals with TBMN may be reviewed every one to 2 years for proteinuria, hypertension and renal impairment by their primary care provider.**

### **Genetic counselling**

TBMN is inherited but the penetrance of hematuria is only 70%.<sup>3</sup> The *de novo* mutation rate is low, and almost all affected individuals have another family member with the causative mutation but not necessarily hematuria.<sup>3</sup> Incompletely penetrant hematuria means that apparently unaffected family members may have affected offspring. On

average, half the children of an individual with TBMN inherit the mutation but fewer have hematuria. The offspring of two parents with TBMN have a 25% risk of autosomal recessive Alport syndrome if both parents have a mutation in the same *COL4A3* or *COL4A4* gene.

**Recommendation 17. All individuals with TBMN and their families should be advised of the diagnosis of TBMN, its inherited nature, and their low risk of renal failure.**

### **Renal transplantation**

There have been many reports of successful cadaveric renal transplants from donors with TBMN.<sup>3,59,65</sup> The risk for live donors with TBMN is less certain since normal donors already have an increased risk of hypertension and microalbuminuria.

**Recommendation 18. Individuals with TBMN may be kidney donors if they have normal levels of proteinuria, blood pressure, and renal function, and if X-linked Alport syndrome and coincidental renal disease have been excluded by genetic testing and renal biopsy. A renal biopsy is mandatory prior to donation to assess renal damage. If an individual with TBMN proceeds with renal donation, he or she must be aware of the risks, and use nephroprotective strategies to minimise the effects of hypertension and proteinuria from the time of surgery.**

### **Pregnancy**

There are normally no increased risks during pregnancy in TBMN if there is no proteinuria, hypertension or renal impairment. Pre-eclampsia is not more common.

### **Conclusions**

There are still unresolved issues in the diagnosis and management of patients with Alport syndrome and TBMN. Randomised controlled trials are expensive, and the results may take years. In the future, we are likely to rely more on the Registries where patients undergo semi-standardised treatment and their clinical progress is updated on-line sometimes by the patients themselves. In addition, the mutation database initiatives will help tease out how mutations in autosomal recessive Alport syndrome and TBMN affect clinical features. Whole genome sequencing is likely to be the diagnostic test of choice

since it examines all 3 Alport genes simultaneously. However, in the meantime, diagnostic laboratories must improve their detection methods to ensure detection of both mutations in autosomal recessive disease. Otherwise patients are misdiagnosed with TBMN or autosomal dominant Alport syndrome where the clinical implications are very different.

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**Table 1: The diagnosis of X-linked Alport syndrome**

<b>Criteria</b>	<b>Sensitivity</b>	<b>Specificity</b>	<b>Comments</b>
Family history of Alport syndrome	High (80%)	High	A positive history will either be obvious immediately or the family will need to spend time asking distant family members. A family history may be absent with <i>de novo</i> disease, where families are small, there is no affected adult male, or disease is atypical
High tone sensorineural hearing loss	High	Moderate	Also occurs with ageing. Hearing loss is also common in other inherited renal diseases, and in renal failure and dialysis
Lenticonus	Low-moderate (30%)	Very high	Only occurs in Alport syndrome. May be misdiagnosed as cataract
Retinal flecks in perimacular region	Moderate (50%)	Very high	The perimacular flecks only occur in Alport syndrome but may be overlooked or misdiagnosed
Lamellated GBM	High	Very high	Typically generalised in affected adult males. Focal in boys and females but progresses with time
$\alpha 3\alpha 4\alpha 5(IV)$ collagen chains absent from GBM	Moderate (80% of males and 60% females)	High	May be focally absent in females
$\alpha 5(IV)$ collagen chain absent from skin	Moderate (80% of males and 60% females)	High	May be focally absent in females
Linkage to the <i>COL4A5</i> locus	>98% (depends on LOD score)	High	Can exclude disease even with only a few affected and unaffected family members.
<i>COL4A5</i> pathogenic variant	High (>90%)	High	May be difficult to distinguish between pathogenic and non-pathogenic variants

**Table 2: Other causes of the characteristic features of Alport syndrome**

Clinical feature	Causes
<p><b>Persistent familial hematuria</b></p>	<p><b>Glomerular hematuria</b></p> <ul style="list-style-type: none"> <li>• Thin basement membrane nephropathy</li> <li>• Familial IgA disease</li> <li>• Alport syndrome</li> <li>• <i>MYH9</i>-related disorders (Fechtner, Epstein syndromes)</li> <li>• Membranoproliferative glomerulonephritis type 2 ('dense deposit disease')</li> <li>• Familial hemolytic uremic syndrome</li> <li>• C3 nephropathy</li> </ul> <p><b>Non-glomerular hematuria</b></p> <ul style="list-style-type: none"> <li>• Autosomal dominant polycystic kidney disease</li> <li>• Sickle cell disease or trait</li> <li>• Familial hypercalciuria, other familial forms of urolithiasis</li> </ul>
<p><b>Renal failure plus hearing loss</b></p>	<ul style="list-style-type: none"> <li>• <i>MYH9</i>-related disorders (Fechtner syndrome)</li> <li>• Nephronophthisis</li> <li>• Bartter syndrome type 4A</li> <li>• Distal renal tubular acidosis</li> <li>• MELAS syndrome</li> <li>• Fabry disease</li> <li>• Branchio-Oto-Renal syndrome</li> <li>• Townes-Brocks syndrome</li> <li>• CHARGE syndrome</li> <li>• Kallmann syndrome</li> <li>• Alstrom disease</li> <li>• Muckle Wells syndrome</li> </ul>
<p><b>Hematuria plus coincidental hearing loss</b></p>	<ul style="list-style-type: none"> <li>• Middle ear infections</li> <li>• Age</li> <li>• Industrial noise exposure</li> <li>• Ototoxic drugs</li> <li>• Dialysis</li> </ul>

<b>Renal failure plus retinal flecks or drusen</b>	<ul style="list-style-type: none"> <li>• Membranoproliferative glomerulonephritis type 2</li> <li>• IgA disease, SLE and some other forms of glomerulonephritis</li> <li>• Severe hypertension ('macular star')</li> <li>• C3 nephropathy</li> </ul>
<b>Lamellated GBM</b>	<ul style="list-style-type: none"> <li>• Focal damage</li> <li>• <i>MYH9</i>-related disorders (Fechtner, Epstein syndromes)</li> <li>• Pierson syndrome</li> <li>• Nail-patella syndrome</li> <li>• Mutations in the tetraspanin (<i>CD151</i>) gene</li> <li>• Frasier syndrome</li> <li>• Galloway-Mowat syndrome</li> </ul>



**Table 3: Distinction between X-linked and autosomal recessive Alport syndrome**

<b>Characteristic</b>	<b>X-linked Alport syndrome</b>	<b>Autosomal recessive Alport syndrome</b>
<b>Prevalence</b>	More common, occurs in 85% of all families	15% of all families
<b>Gender</b>	Males are affected more often and more severely than females	Males and females affected with equal frequency and severity. Suspected where a female has renal failure or lenticonus
<b>Age at first presentation</b>	Males have hematuria from infancy, but renal failure occurs typically from the teenage years onwards	Males and females present with hematuria from infancy, and develop renal failure in childhood or adult life
<b>Family history of renal failure</b>	Other male relatives may have renal failure. Disease appears to 'skip' a generation because affected females are much less likely to develop renal failure	Renal failure often found in only one generation (except in rare multiply-consanguineous families)
<b>Carrier features</b>	95% affected females have hematuria and 15% develop renal failure by the age of 60 years. Hearing loss and peripheral retinopathy occur in nearly half by the age of 60	Carriers often have hematuria, but renal failure is uncommon, and hearing loss, lenticonus and retinopathy do not occur
<b>Pedigree analysis</b>	Mother typically has hematuria and father to son disease transmission does not occur	Hematuria but not renal failure may be present in the mother and father, and in other family members.
<b>Lamellated GBM</b>	Yes but thinning with focal lamellation in young boys and females that becomes more lamellated with time	Yes
<b><math>\alpha3\alpha4\alpha5(IV)</math> collagen chains absent from GBM</b>	Yes	Yes, but the $\alpha5(IV)$ chain persists in Bowman's capsule and the distal tubular basement membrane
<b><math>\alpha5(IV)</math> collagen chain absent from skin</b>	Yes	No, the $\alpha5(IV)$ chain persists in the skin
<b>Mutation analysis</b>	A single pathogenic variant in the <i>COL4A5</i> gene	Two pathogenic variants in either the <i>COL4A3</i> or <i>COL4A4</i> gene on different chromosomes

**Table 4: Indications for genetic testing in Alport syndrome**

- To confirm the diagnosis of Alport syndrome
- To identify the mode of inheritance. This indicates the risk of renal failure for other family members
- To exclude Thin basement membrane nephropathy in individuals with persistent hematuria
- To help predict the risk of early onset renal failure in X-linked disease based on DNA mutation characteristics or a previously-reported association.
- To enable early prenatal diagnosis for females at risk of an affected pregnancy.
- To predict whether an embryo is affected prior to implantation ('pre-implantation genetic diagnosis')

### **Table 5: Relevant websites including those for Patient Support Groups**

[alportsyndrome.org](http://alportsyndrome.org). This is the website of the US Alport Foundation. It is linked to ASTOR and has contact details for genetic testing laboratories worldwide but only for kidney and skin immunohistochemistry in N America

[www.actionforalportscampaign.org](http://www.actionforalportscampaign.org) UK based patient support group that provides information for families affected by Alport syndrome and brings together professionals with a particular interest in the condition.

Alport Syndrome Treatments and Outcomes Registry (ASTOR) – patient registry. This is a voluntary international patient registry established at the University of Minnesota. ASTOR aims to provide patients and families with the most current information about Alport syndrome and information from this Registry will be used to design future treatments.

Alport. Orphanet. This mainly Euro-centric site describes the disease classifications, recent medical publications, is linked to other relevant websites, gives contact details for expert treating centres, diagnostic testing laboratories, Patient Support Groups, Funding groups, research projects, clinical trials, registries or biobanks and networks

There are 2 currently 2 curated *COL4A5* mutation databases: LOVD ([grenada.lumc.nl/LOVD/COL4A/](http://grenada.lumc.nl/LOVD/COL4A/)) and ARUP ([www.arup.utah.edu/database/Alport](http://www.arup.utah.edu/database/Alport)) and

NCBI Genes and Disease – Alport syndrome – a useful and comprehensive overview of Alport syndrome with specific genetic information clearly spelled out for each type of inheritance

[www.alport.de](http://www.alport.de) European therapy registry. This is a voluntary international patient registry established at the University of Goettingen, Germany

[www.alport.de/EARLY](http://www.alport.de/EARLY) PRO-TECT. This website has information about the phase III, multicenter, randomised placebo-controlled double-blinded trial to investigate the optimal timing of ACE inhibitor therapy and its safety in paediatric patients with early stage disease

**Table 6: The diagnosis of Thin basement membrane nephropathy**

<b>Characteristic feature</b>	<b>Sensitivity</b>	<b>Specificity</b>	<b>Comments</b>
Persistent glomerular hematuria, minimal proteinuria, normal blood pressure, and normal renal function.	High (80%)	Moderate	TBMN is the commonest cause. Occurs in IgA disease too but often higher urinary RBC counts and proteinuria less commonly
Family history of hematuria	Moderate (67%)	High	However a family history of hematuria is also common in X-linked Alport syndrome
Generally thinned GBM without focal lamellation	95%	High	
Collagen IV $\alpha 3\alpha 4\alpha 5$ network present in GBM	100%	moderate	Supports but does not prove the diagnosis of TBMN
$\alpha 5(IV)$ collagen chain present in skin			Supports but does not prove the diagnosis of TBMN
Hematuria segregates with <i>COL4A3/COL4A4</i>	40%	High	
Hematuria does not segregate with the <i>COL4A5</i> locus	High	High	Linkage studies require careful characterisation of other family members but are possible with very few members
Single mutation in <i>COL4A3</i> or <i>COL4A4</i>	80%	Very high	

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