Expert Guidelines for the Management of Alport Syndrome and Thin Basement Membrane Nephropathy

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ABSTRACT

Few prospective, randomized controlled clinical trials address the diagnosis and management of patients with Alport syndrome or thin basement membrane nephropathy. Adult and pediatric nephrologists and geneticists from four continents whose clinical practice focuses on these conditions have developed the following guidelines. 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 (Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees—U.S. Preventive Services Task Force) evidence. 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 most mothers of affected males; the treatment of males with X-linked Alport syndrome and individuals with autosomal recessive disease with renin-angiotensin system blockade, 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 recognize that as evidence emerges, including data from patient registries, these guidelines will evolve further.

Glomerular hematuria that persists for at least a year occurs in at least 1% of the population[1–3] and is typically due to thin basement membrane nephropathy (TBMN). Much less often, it results from Alport syndrome.[3–6] 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 the rate of deterioration.

Alport syndrome is characterized by hematuria, renal failure, hearing loss, lenticonus, and retinal flecks;[7] a lamellated glomerular basement membrane (GBM)[8] with an abnormal collagen IV composition;[9] and mutations in the *COL4A5* or *COL4A3/COL4A4* genes.[10,11] Eighty-five percent of families have X-linked inheritance with mutations in *COL4A5*,[12] and most of the others have autosomal recessive disease with homozygous or compound heterozygous mutations in both copies (*in trans*) of *COL4A3* or *COL4A4*.[11] Autosomal dominant inheritance is very rare and results from heterozygous *COL4A3* or *COL4A4* variants.[13]

Individuals with TBMN have isolated hematuria.[3] TBMN is usually caused by heterozygous *COL4A3* or *COL4A4* mutations and often represents the carrier state of autosomal recessive Alport syndrome.[3]

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

Here we define Alport syndrome and TBMN, provide a diagnostic algorithm for the patient with persistent hematuria, describe the clinical features in Alport syndrome and how they contribute to the likelihood of this diagnosis, list diseases that share clinical features with Alport syndrome, and discuss criteria that help distinguish between X-linked and autosomal recessive inheritance.

The following recommendations describe the use of the terms "Alport syndrome" and "TBMN" (recommendation 1); criteria for the diagnosis of Alport syndrome (recommendation 2); the

distinction between X-linked and autosomal recessive inheritance (recommendation 3); how to predict the clinical phenotype from the *COL4A5* mutation (recommendation 4); the importance of identifying other affected family members (recommendation 5); the uses of genetic counseling (recommendation 6); ongoing medical management (recommendation 7); issues for the transplant recipient (recommendation 8); and the affected female: diagnosis, management, and the risks of renal donation (recommendation 9). The recommendations also address autosomal recessive Alport syndrome: family screening (recommendation 10), genetic counseling (recommendation 11); management (recommendation 12), and renal donation (recommendation 13). The recommendations for TBMN include the criteria for diagnosis (recommendation 14), genetic testing (recommendation 15), management and prognostic indicators (recommendation 16), family screening (recommendation 17), and renal donation (recommendation 18).

Prospective randomized controlled clinical trials for the diagnosis and management of Alport syndrome and TBMN are difficult to undertake because of the small numbers of patients at individual treatment centers and their different stages of disease at presentation. Instead, our recommendations are largely based on the experience and opinions of the authors, as well as retrospective studies in humans, animal experiments, and analysis of the Alport registries. The authors were able to reach consensus on all the recommendations and considered that the benefits outweighed any potential risks. The authors were guarded only in suggesting the time to introduce renin-angiotensin system blockade in X-linked Alport syndrome before formal evaluation in clinical trials. The evidence for all the recommendations is presented in the introductory comments to 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 hearing loss, lenticonus, or retinopathy. The term "TBMN" should not be used in females or boys with a thinned GBM due to X-linked Alport syndrome. Their biopsy specimens, or those of affected family members, usually show a GBM with stretches of splitting or lamellation. Further clinical or genetic testing may be required. Clinicians should remember that inherited hematuria and renal failure may be caused by TBMN with coincidental renal disease.[14]

Recommendation 1

The term "Alport syndrome" should be reserved for patients with the characteristic clinical features and a lamellated GBM with an abnormal collagen IV composition, and in whom a *COL4A5* mutation (X-linked disease) or two *COL4A3* or two *COL4A4* mutations *in trans* (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 the usually benign prognosis seen with TBMN. 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, based on its prevalence, together with a coincidental renal disease, such as IgA GN, or to autosomal recessive Alport syndrome, with a second, undetected mutation. In these circumstances, the correct diagnosis may require further discussions among the nephrologist, pathologist, clinical geneticist, ophthalmologist, and audiologist, and interpretation of the relevant test results.

DIAGNOSIS OF ALPORT SYNDROME

Alport syndrome is suspected when there is persistent glomerular hematuria. The likelihood increases with a family history of Alport syndrome or renal failure, and no other obvious cause; or when the characteristic clinical features (hearing loss, lenticonus, or retinopathy) are present, or the GBM lacks the collagen IV $\alpha3$, $\alpha4$, and $\alpha5$ chains (Figure 1). The diagnosis is confirmed if there is a lamellated GBM or a pathogenic mutation in the *COL4A5* gene or two pathogenic *COL4A3* or *COL4A4* mutations. The sensitivity and specificity of each of these features for X-linked Alport syndrome are provided in Table 1.[15] Genetic testing is at least 90% sensitive for X-linked disease.[16]

Alport syndrome must be distinguished from the other causes of inherited hematuria and renal failure, inherited renal disease and hearing loss, retinal flecks, and GBM lamellation (Table 2). Hematuria is not typical of the most common familial forms of pediatric renal failure, namely FSGS and nephronophthisis. Hearing loss occurs with many different inherited renal diseases but for other reasons. Other causes of GBM lamellation are very rare or have further distinctive histological 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, an ophthalmologic examination, and, preferably, renal biopsy for GBM ultrastructure, collagen IV composition, and an assessment of damage. The diagnosis of Alport syndrome is highly likely if there are glomerular hematuria and a family history of Alport syndrome with no other cause for the hematuria ; if bilateral high-tone sensorineural hearing loss, lenticonus, or fleck retinopathy is present; or if the GBM lacks the collagen IV $\alpha5$ chain. The
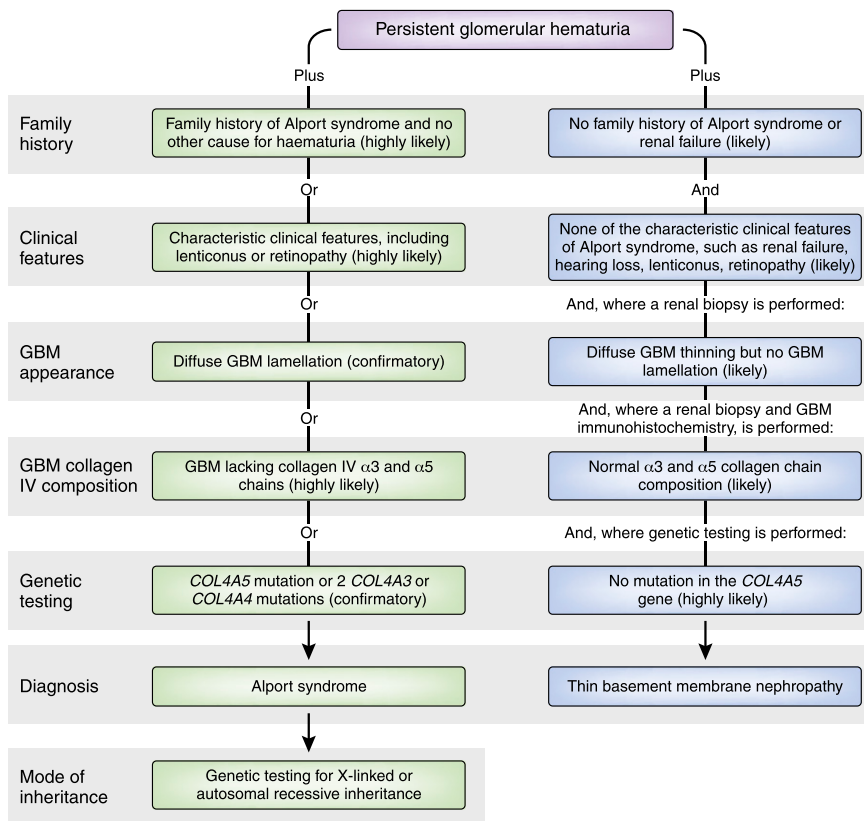
Figure 1. An approach to distinguish between Alport syndrome and thin basement membrane nephropathy as the cause of persistent glomerular hematuria.

diagnosis of Alport syndrome is confirmed with the demonstration of a lamellated GBM or a *COL4A5* or two *COL4A3* or *COL4A4* mutations. In individuals in whom 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 same strategy.

MODES OF INHERITANCE

Once Alport syndrome has been diagnosed, 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.[12] The mode of inheritance is sometimes suspected from the pedigree. With X-linked inheritance, disease appears to skip a generation, where there is an affected female with hematuria and no other features. With recessive inheritance, disease typically occurs in a single generation, males and females are affected equally often and equally severely, and the father of an affected individual may have hematuria. Recessive inheritance is also suspected where a young female has renal failure, hearing loss, and ocular abnormalities. Inheritance is usually confirmed with genetic testing. Sometimes the GBM collagen IV composition is used; however, this test is not widely available, and interpretation of the results may be difficult in females with X-linked disease.[17] Features that distinguish X-linked from autosomal recessive Alport syndrome are summarized 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 because only 15% mutations occur *de novo* and penetrance is 95%.[18] 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 age 30 years usually have extrarenal manifestations,[15,19] 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 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 widespread in men. The GBM is initially thinned in boys, but there is focal lamellation[20] that becomes more extensive with time. The GBM collagen IV composition is typically abnormal and lacks the $\alpha3\alpha4\alpha5$ network.[9,21] The epidermal membrane also has no $\alpha5$ chain,[22] and examination of a skin biopsy specimen is less invasive and the results may be available sooner than assessment of a renal biopsy sample.[22]

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 children for Alport syndrome are outweighed by the potential of treatment to delay end-stage renal failure.[23] Further information on molecular testing for Alport syndrome is available at www.genereviews.org (Table 5).

Table 1. The diagnosis of X-linked Alport syndrome

Criteria	Sensitivity	Specificity	Comments
Family history of Alport syndrome	High (80%)	High	A positive history will be obvious immediately, or the family will need to spend time asking distant family members. A family history may be absent with *de novo* disease or where families are small, there is no affected adult male, or disease is atypical.
Bilateral high-tone sensorineural hearing loss	High	Moderate	Also occurs with aging, middle ear infections, and industrial noise exposure. Hearing loss is also common in other inherited renal diseases and with renal failure and dialysis.
Lenticonus	Low to moderate (30%)	Very high	Only occurs in Alport syndrome. May be misdiagnosed as cataract.
Central fleck retinopathy	Moderate (50%)	Very high	The perimacular flecks occur only in Alport syndrome but may be overlooked or misdiagnosed.
Lamellated GBM	High	Very high	Typically generalized in affected adult males. Focal in boys and females but progresses with time.
$\alpha3\alpha4\alpha5$(IV) collagen chains absent from GBM	Moderate (80% of males and 60% females)	High	May be focally absent in females.
$\alpha5$(IV) collagen chain absent from skin	Moderate (80% of males and 60% females)	High	May be focally absent in females.
COL4A5 pathogenic variant	High (>90%)	High	May be difficult to distinguish between pathogenic and nonpathogenic variants.

The mutation detection rate in X-linked Alport syndrome is at least 90% with a combined approach of sequencing genomic DNA or hair-root or skin cDNA, followed by multiplex ligation-dependent probe amplification to detect large deletions, insertions, or duplications.[24–28] Current techniques identify mainly coding region variants. Mutations are more likely to be identified in individuals with early-onset renal failure and extrarenal features,[15,19,29] because the diagnosis of Alport syndrome is more likely to be accurate.

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).[16] Clinical features depend mainly on the mutation's location and genotype. About 50% result in a stop codon either directly or downstream, and 40% of mutations are missense.[16] Large deletions and rearrangements, nonsense mutations, and carboxy terminal missense mutations typically result in early-onset renal failure, hearing loss, and ocular abnormalities,[15,29] whereas amino terminal missense mutations are often associated with late-onset renal failure without the extrarenal features. The likelihood of early-onset renal failure in affected males can also be predicted from the effect of the mutation in other affected male relatives.[29]

Genetic linkage studies are used rarely to exclude a mode of inheritance in families where no mutation has been demonstrated,[17] and, sometimes, in prenatal or preimplantation genetic diagnosis where the mutation is not known.[30] 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, indeed, 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 help predict the likelihood of early-onset renal failure and extrarenal features. These are sometimes already obvious from the disease manifestations in 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. For any female with X-linked disease, each of her sons has a 50% risk of being affected and developing renal failure, and each of her daughters has a 50% risk of being affected. In contrast, a male with X-linked disease can be reassured that none of his sons will inherit the mutation, but all of his daughters, and half of her sons and daughters, will be affected. Thus, overall, the immediate risks are greater for the offspring of an affected female than for a male with X-linked disease.

In any family with X-linked Alport syndrome, individuals with hematuria are highly likely to be affected, but other coincidental causes of 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. Most

Table 2. Other causes of the characteristic features of Alport syndrome

Clinical Feature	Causes
Persistent familial hematuria	Glomerular hematuria
	TBMN
	Familial IgA disease
	MYH9-related disorders (Fechtner, Epstein syndromes)
	Membranoproliferative GN type 2 (dense deposit disease)
	Familial hemolytic uremic syndrome
	C3 nephropathy
	Nonglomerular hematuria
	Autosomal dominant polycystic kidney disease
	Sickle cell disease or trait
	Familial hypercalciuria, other familial forms of urolithiasis
Renal failure plus hearing loss	*MYH9*-related disorders (Fechtner syndrome)
	Nephronophthisis
	Bartter syndrome
	Distal renal tubular acidosis
	MELAS syndrome
	Fabry disease
	Branchio-oto-renal syndrome
	Townes-Brock syndrome
	CHARGE syndrome
	Kallmann syndrome
	Alstrom disease
	Muckle-Wells syndrome
Hearing loss	Middle-ear infections
	Age
	Industrial noise exposure
	Ototoxic drugs
	Renal failure, dialysis
Retinal flecks	Membranoproliferative GN type 2
	IgA disease, systemic lupus erythematosus, and some other forms of GN
	Severe hypertension (macular star)
	C3 nephropathy
Lamellated GBM	Focal damage
	MYH9-related disorders (Fechtner, Epstein syndromes)
	Pierson syndrome
	Nail-patella syndrome
	Mutations in the tetraspanin (*CD151*) gene
	Frasier syndrome
	Galloway-Mowat syndrome

MELAS, mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke; CHARGE, coloboma, heart anomalies, choanal atresia, retardation of growth and development, and genital and ear anomalies.

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 Counseling

Genetic counseling is usually appropriate where available.

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 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 α5 chain all correlate with an increased likelihood of early-onset renal failure in males,[18,30,31] 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.[32] The retinopathy progresses but does not affect vision or require treatment.

Angiotensin-converting enzyme (ACE) inhibitors reduce proteinuria in children with X-linked Alport syndrome.[33] Angiotensin-receptor blockers and aldosterone inhibitors have additional benefits for proteinuria.[34] 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 men, even when begun before the onset of proteinuria.[35] However, it is critical that the effect of renin-angiotensin blockade on proteinuria and renal failure progression is formally evaluated (EARLY PRO-TECT Alport study, EU Clinical Trials Register).[36] In the meantime, one approach is to target individuals at greatest risk of early-onset renal failure.[37] Other potential therapies include statins,[38] metalloproteinase inhibitors,[39] vasopeptidase inhibitors,[40] chemokine receptor antagonists,[41] and stem cell therapy.[42,43]

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

Characteristic	X-linked Alport Syndrome	Autosomal Recessive Alport Syndrome
Prevalence	More common, occurs in 85% of all families	15% of all families
Sex	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, hearing loss, or ocular abnormalities
Age at first presentation	Males have hematuria from infancy, but renal failure occurs typically from the teenage years	Males and females present with hematuria from infancy and develop renal failure in childhood or adult life
Family history of renal failure	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 typically found in only one generation. The exceptions are the rare families with multiple examples of consanguinity
Carrier features	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 age 60 yr	Carriers often have hematuria, but renal failure is uncommon, and hearing loss and ocular abnormalities do not occur
Pedigree analysis	Mother typically has hematuria, and the father has no hematuria, that is, 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.
Lamellated GBM	Yes, but thinning with focal lamellation in young boys and females becomes more lamellated with time	Yes
$\alpha3\alpha4\alpha5(IV)$ collagen chains absent from GBM	Yes	Yes, but the $\alpha5(IV)$ chain persists in the Bowman capsule and the distal tubular basement membrane
$\alpha5(IV)$ collagen chain absent from skin	Yes	No, the $\alpha5(IV)$ chain persists in the skin
Mutation analysis	A single pathogenic mutation in the COL4A5 gene	Two pathogenic mutations in the COL4A3 or COL4A4 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 TBMN 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 previously reported associations

To enable early prenatal diagnosis for females at risk of an affected pregnancy

To predict whether an embryo is affected prior to implantation (preimplantation genetic diagnosis)

Recommendation 7

Males with X-linked Alport syndrome should be managed lifelong by a nephrologist and have their risk factors for progressive renal failure optimized, including careful management of 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 minimize further hearing loss.

Renal Transplantation

Patients with X-linked Alport syndrome who undergo transplantation have survival rates and graft survival rates similar to or better than those of patients with other inherited renal diseases.[44,45] Affected female family members should be strongly discouraged from donating a kidney, but where this has occurred, both the donor and the recipient should receive nephroprotective treatment, such as renin-angiotensin system blockade, from the time of surgery.

Three percent to 5% of males develop anti-GBM disease with rapid allograft loss after transplantation.[31,46,47] Anti-GBM disease is more common with large gene deletions[48] but also occurs with other mutations.[31] In these individuals, the risk of anti-GBM disease is higher after subsequent renal transplants, and anti-GBM antibodies are best demonstrated with GBM immunohistochemistry and less effectively with anti-GBM ELISA because of different epitope specificities.[49]

Recommendation 8

Males with X-linked Alport syndrome and increased risk of anti-GBM 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 renal impairment.

Table 5. Relevant websites, including those for patient support groups

Web site	Description
www.alportsyndrome.org	This is the website of the US Alport Foundation. It is linked to the Alport Syndrome Treatments and Outcomes Registry (ASTOR) and has contact details for genetic testing laboratories worldwide and for kidney and skin immunohistochemistry in North America.
www.actionforalportscampaign.org	United Kingdom–based patient support group that provides information for families affected by Alport syndrome and brings together professionals with a particular interest in the condition.
www.alportregistry.org	ASTOR is a voluntary international patient registry established at the University of Minnesota that aims to provide patients and families with the most current information about Alport syndrome. Information from this registry will be used to design future treatments.
www.orpha.net/consor/cgi-bin/ OC_Exp.php?Expert=63&lng=EN	Alport Orphanet is a mainly European-centric site that describes the disease classifications; lists recent medical publications; links to other relevant websites; and gives contact details for expert treating centers, diagnostic testing laboratories, patient support groups, funding groups, research projects, clinical trials and registries, biobanks and networks.
www.arup.utah.edu/database/ALPORT/ ALPORT_welcome.php www.grenada.lumc.nl/LOVD2/COL4A/ home.php?select_db=COL4A3	These curated *COL4A5* mutation databases are hosted by the LOVD and ARUP Laboratories.
www.ncbi.nlm.nih.gov/books/NBK22183	This useful and comprehensive overview of Alport syndrome from NCBI provides specific genetic information clearly described for each form of inheritance.
www.alport.de	This is a voluntary international patient registry established at the University of Goettingen, Germany.
www.alport.de/EARLY PRO-TECT	This website has information about the phase III, multicenter, randomized, double-blind, placebo-controlled trial to investigate the optimal timing of ACE inhibitor therapy and its safety in pediatric patients with early-stage disease.

ASTOR, Alport Syndrome Treatments and Outcomes Registry; LOVD, Leiden Open Variation Database; NCBI, National Center for Biomedical Information.

X-linked Alport Syndrome in Females

Almost all (95%) females with X-linked Alport syndrome have hematuria, and many eventually develop other clinical features, especially proteinuria (75%),[18] end-stage renal failure (8%–30%, overall 15%, by the age of 60), hearing loss (40%), or peripheral retinopathy (40%).[19,50] Lenticonus may not occur, and central retinopathy is rare. It is therefore debatable whether females should be considered affected or carriers. Those who prefer the term "affected" maintain that 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 diagnosed only 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.[51] The collagen IV $\alpha 3\alpha 4\alpha 5$ network is patchily present depending on X chromosome inactivation.

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.[52] Again preliminary support, but no evidence, suggests a beneficial effect for the initiation of ACE inhibitor treatment even before the onset of proteinuria.[35] Poor prognostic markers in females include episodes of macroscopic hematuria in childhood and proteinuria.[53–55] A renal biopsy is warranted if there is significant proteinuria (for example, >1 g/d in adults) or renal impairment. However, changes in the renal biopsy specimen and GBM may be patchy, sampling variation is common, and interpretation may be difficult.[56] Sometimes females with X-linked Alport syndrome themselves require a transplant for renal failure, but they do not subsequently develop anti-GBM disease.[30]

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 50% if her mother is 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.[57] A kidney biopsy is mandatory in a mutation-carrying potential donor, even in those with normal renal function and normal levels of proteinuria, to assess renal damage resulting from the effects of random X inactivation.[54] Female carriers should only be kidney donors of last resort.[58] 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, preferably, genetic testing to formally exclude the diagnosis of X-linked Alport syndrome.

The risk of preeclampsia is increased in affected females with hypertension, proteinuria, or renal impairment, and pregnancy may accelerate any decline in renal function already present.[59] Preexisting hypertension and renal impairment predict an increased risk of obstetric complications,[60] and proteinuria, hypertension, and renal impairment are all associated with preterm delivery.[60]

Recommendation 9
Female carriers of X-linked Alport syndrome typically have a good renal outcome, but, on average, 15% develop end-stage renal failure by the age of 60 years. Thus, the carrier state should be viewed as at-risk rather than a benign condition. Clinicians should endeavor to convey this information in a way that encourages regular follow-up examinations for signs of progression, such as the development of hypertension, proteinuria, or renal impairment, and for hearing loss, without engendering undue anxiety. Some women with hematuria 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 have clinical manifestations. 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 family's mutation is known, 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 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. Predonation kidney biopsy is mandatory 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 minimize the effects of hypertension and proteinuria from the time of surgery. Fifteen percent 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 and occurs in a single generation or a consanguineous family, where males and females in a family are affected with equal frequency and severity, where the father also has hematuria, or where a female has renal failure, hearing loss, or ocular abnormalities. Autosomal recessive inheritance is confirmed when there are two *COL4A3* or two *COL4A4* pathogenic mutations or the GBM lacks the collagen IV $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains but the $\alpha 5$ chain persists in the Bowman capsule and the distal tubular and the epidermal membrane.[21,61]

Genetic Testing
Genetic testing is useful to confirm the diagnosis of autosomal recessive Alport syndrome[16] when 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 the *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.

Genetic Counseling
Individuals with autosomal recessive Alport syndrome are typically from a single generation within a family, but the situation is more complicated where the family includes multiple examples of consanguinity. The risk of the sibling 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 10
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, and the risks for other family members. A nondirective 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 and 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.

Recommendation 11
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.

Monitoring and Treatment
Evidence from a small retrospective registry analysis suggests that renin-angiotensin system blockade, for example with ACE inhibitors, delays renal failure and improves life expectancy in individuals with autosomal recessive Alport syndrome and may improve the outlook in carriers.[35,52]

Recommendation 12
Individuals with autosomal recessive Alport syndrome should be managed by a nephrologist and have their risk factors for progressive renal failure optimized, including 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 minimize further hearing loss.

Renal Donation
Individuals with only one of the mutations that contribute to autosomal recessive Alport syndrome (parents, offspring, some siblings) have a phenotype identical to that of TBMN. They can usually be kidney donors if a predonation renal biopsy excludes significant renal damage and genetic testing excludes X-linked Alport syndrome.[3,62]

Recommendation 13
Individuals from families with autosomal recessive Alport syndrome who have only one of the causative mutations (parents, offspring, some siblings) may be renal donors if they have normal BP, proteinuria levels, and renal function; if coincidental renal disease has been excluded by renal biopsy; and if X-linked Alport syndrome has been excluded by genetic testing.

TBMN

TBMN affects 1% of the population and is characterized by hematuria, proteinuria ($\leq$200 mg/L), normal BP, normal renal function, and a thinned GBM (Table 6).[3] TBMN usually represents the carrier state for autosomal recessive Alport syndrome, and inheritance is autosomal dominant. Typically the prognosis is good, but there is also an increased risk of hypertension, proteinuria, and renal impairment.[3] The risk of renal failure is increased if there is coincidental renal disease or diabetes.[3] It remains important to exclude X-linked Alport syndrome in these patients.[3]

Diagnosis
TBMN is 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 <250 nm or a measurement specific to a laboratory and adjusted for age and sex.[63] This thinning involves at least 50% of the GBM, without the lamellation found in Alport syndrome. However, the Alport lamellation may be patchy, and occasionally errors are made in basing a diagnosis on GBM appearance, especially in boys and females.

The demonstration of normal expression of the collagen IV α3, α4, and α5 chains in renal basement membranes in patients whose clinical features are otherwise consistent with TBMN supports this diagnosis.[64]

Heterozygous COL4A3 and COL4A4 mutations also cause autosomal dominant Alport syndrome.[65] The diagnosis of autosomal dominant Alport syndrome is reserved for individuals with a lamellated GBM and autosomal dominant inheritance. Some reports of autosomal dominant Alport syndrome are likely to represent TBMN with a coincidental renal disease, such as IgA GN.[14] Errors in which the diagnosis is actually TBMN mean 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, normal levels of proteinuria, and normal BP 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 suspected of having TBMN should undergo renal biopsy if they have atypical features (proteinuria in adults >1.0 g/d or renal impairment [estimated GFR < 90 ml/min per 1.73 m^2]), 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 both the COL4A3 and the COL4A4 genes is usually required. This is labor-intensive and expensive, and it is usually more important in an individual with hematuria only to exclude a COL4A5 mutation and hence X-linked Alport syndrome, rather than to make a positive molecular diagnosis of TBMN.

Table 6. Diagnosis of TBMN

Characteristic Feature	Sensitivity	Specificity	Comments
Persistent glomerular hematuria, minimal proteinuria, normal BP, and normal renal function	High (80%)	Moderate	TBMN is the most common cause; occurs in IgA disease too but often with higher urinary red blood cell counts and (less commonly) proteinuria
Family history of hematuria	Moderate (70%)	High	Family history of hematuria is also common in X-linked Alport syndrome
Generally thinned GBM without focal lamellation	95%	High	
Collagen IV α3α4α5 network present in GBM	100%	Moderate	Supports but does not prove the diagnosis of TBMN
α5(IV) collagen chain present in skin			Supports but does not prove the diagnosis of TBMN
Hematuria segregates with COL4A3/COL4A4	40%	High	
Hematuria does not segregate with the COL4A5 locus	High	High	Linkage studies require careful characterization of other family members but are possible with very few members
Single mutation in COL4A3 or COL4A4	80%	Very high	

Recommendation 15

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

Monitoring and Treatment

The prognosis of TBMN is usually good.[3] 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 nondiabetic renal disease that renin-angiotensin blockade delays progression to end-stage renal failure in at-risk individuals.[52,66–68]

Recommendation 16

Individuals with TBMN should be assessed at presentation for poor prognostic indicators (hypertension, proteinuria, renal impairment). Those with these features should be managed by a nephrologist, and treatment should include an ACE inhibitor to delay the onset of renal failure. Other individuals with TBMN may be reviewed every 1–2 years for hypertension, proteinuria, and renal impairment by their primary care provider.

Genetic Counseling

TBMN is inherited, but the penetrance of hematuria is only 70%.[3] The *de novo* mutation rate is low, and almost all affected individuals have another family member with the causative mutation, but not necessarily hematuria.[3] On average, half the children of an individual with TBMN inherit the causative mutation but, because hematuria is incompletely penetrant, 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.[3,62,69] The risk for live donors with TBMN is less certain because normal donors already have an increased risk of hypertension and microalbuminuria.

Recommendation 18

Individuals with TBMN may be kidney donors if they have normal BP, proteinuria, and renal function, and if genetic testing and renal biopsy have excluded X-linked Alport syndrome and coincidental renal disease. 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 minimize the effects of hypertension and proteinuria from the time of surgery.

Pregnancy

Risks are not usually increased during pregnancy in women with TBMN if hypertension, proteinuria, and renal impairment are not present. Preeclampsia is not more common.

CONCLUSIONS

There are still unresolved issues in the diagnosis and management of patients with Alport syndrome and TBMN. Randomized controlled trials are expensive, and the results may take years. In the future, we are likely to rely more on registries, in which patients undergo semi-standardized treatment and their clinical progress is updated online, sometimes by the patients themselves. In addition, the mutation database initiatives will help explain how mutations in autosomal recessive Alport syndrome and TBMN affect clinical features. In

the future, whole genome sequencing is likely to be the diagnostic test of choice because it examines all three Alport genes simultaneously. In the meantime, diagnostic laboratories must improve their methods to ensure detection of both mutations in autosomal recessive disease. Otherwise, TBMN or autosomal dominant Alport syndrome is diagnosed, conditions in which the clinical implications are very different.

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DISCLOSURES

J.S., M.G., O.G., J.D., and F.F. have no conflicts of interest to declare.

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