Genetic Disorders of the Glomerular Basement Membrane

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Contents

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Learning Objectives

1. A moderately detailed understanding of the second most common inherited cause of end-stage renal failure.
2. Understand when to implement treatments to slow its progression.
3. Recognise the possibility of COL4A3-5 mutations in CKD of unknown cause or unrecognised in other patients.
4. Understand uncertain risk to those carrying a normal copy of a COL4A3-5 gene alongside a copy with a significant mutation.

Basement membranes are specialised matrices found beneath epithelial and endothelial cell layers in all organs of the body. In the kidney, the glomerular basement membrane (GBM) forms part of the barrier between blood and filtrate.

Alport syndrome is the second most common genetic cause of renal failure. Blocking angiotensin effects seems to slow its progression. Thin basement membrane nephropathy is a common diagnosis in patients presenting with microscopic haematuria. This chapter reviews our current understanding of these conditions and mentions other less common inherited diseases of the GBM.

62.1 The Glomerular Basement Membrane: Components, Structure and Function

The GBM is part of the glomerular filtration barrier and lies between two layers of cells [1]. It is flanked on one side by endothelial cells that face the glomerular capillary lumen, whilst podocyte foot processes line the other side protruding into the urinary space. The GBM is thicker than other basement membranes, measuring 300–350 nm. In health, the function of the glomerular filtration barrier is to allow the passage of water and small solutes whilst preventing the passage of large proteins.

All basement membranes have four major protein components: (1) type IV collagen, (2) laminin, (3) nidogen and (4) heparan sulphate proteoglycans. Diseases occurring as a result of type IV collagen and laminin gene mutations have been described.

Type IV collagen is the major component of mammalian basement membranes [1], and abnormal type IV collagen is the culprit in most of the inherited GBM disease as we currently identify them.

There are six α chains, and each molecule of type IV collagen is composed of three of these chains. A triple helical structure is common to all collagens, but type IV collagens are characterised by non-collagenous interruptions in the helical structure and retention of non-collagenous domains at each end.

The six α chains (α1(IV) to α6(IV)) are encoded by *COL4A1* to *COL4A6* genes which are arranged in pairs as shown in Fig. 62.1. Each chain has a molecular weight of over 160,000, and the genes encoding them are large and complex.

The composition of the type IV molecule varies between different membranes. An α1-α2 (112) network is common to all basement membranes. The glomerular basement membrane in adults is mainly made from a network of α3α4α5 (345) molecules, which is also present in the eye, ear and lungs (Fig. 62.2). A third network, found in Bowman's capsule, skin and other locations, contains α5-α6 molecules. The 112 network is the first formed during development, so that a later developmental switch is required to form the mature composition. In developing glomeruli, immature nephrons swap from the 112 network to the 345 network as the capillary loops form.

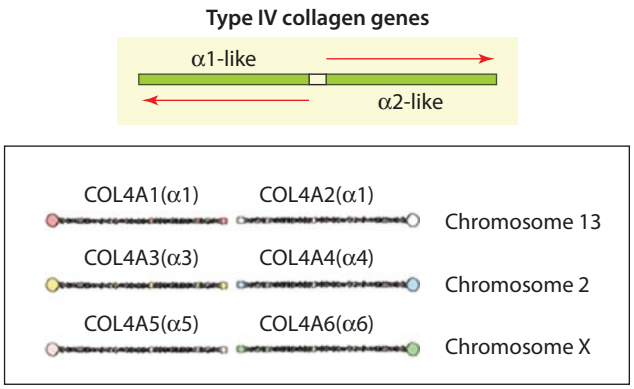

Fig. 62.1 The six type IV collagen genes and their chromosomal relationships. (Reproduced with permission from Neil Turner and ► www.edren.org)

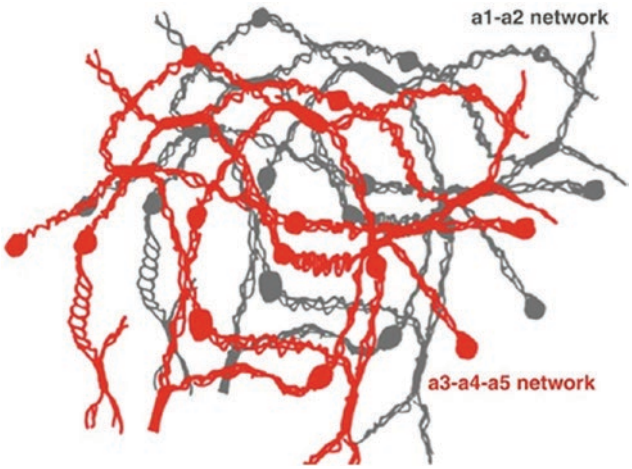

Fig. 62.2 Type IV collagen networks. The α3-α4-α5 is the major network in the GBM. (Reproduced with permission from Neil Turner and ► www.edren.org)

Laminins are large glycoproteins, made of one α, one β and one γ chain from the products of 5 *LAMA*, 4 *LAMB* and 3 *LAMC* genes. Laminin 521 (formed of the α5, β2 and γ1 chains) is the dominant component in adult GBM [1], but like collagen isoforms, this shifts developmentally, from 111 through 511 to the 521 network.

Laminins form a separate network in basement membranes that seems to assemble before the collagen network [1].

Nidogen (also known as entactin) is another universal component of basement membranes. It is a dumbbell-shaped molecule with two isoforms, nidogen 1 and 2, which can bind to both laminin and collagen. *NID* mutations have not yet been identified in human diseases, but deletion of both isoforms is lethal perinatally in mice and associated with abnormal basement membrane development [2].

The heparan sulphate proteoglycan (HSPG) *agrin* is the major HSPG in adult GBM. HSPGs are strongly anionic, giving an electronegative charge to GBM which has been thought to be functionally important. However, *AGRN* (agrin) mutations are associated with a congenital myasthenic syndrome but not renal disease.

Major conditions affecting the GBM are shown ◘ Table 62.1. This chapter considers only genetic causes.

Alport syndrome and thin basement membrane nephropathy are relatively common in renal practice. There is also a handful of rarer diseases.

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62.2 Alport Syndrome

Is the prototypical basement membrane disease. In 1927, Cecil Alport described the condition that now bears his name, in a family with hereditary nephritis and deafness [3, 4].

◘ **Table 62.1** Diseases of the glomerular basement membrane

Inherited diseases of GBM	Acquired diseases of GBM
Alport syndrome	Anti-GBM (Goodpasture) disease
Thin basement membrane nephropathy	Fibrillary nephritis – deposition
Nail-patella syndrome	Inflammatory nephritis – holes in GBM
Pierson syndrome	
HANAC	

62.2.1 Epidemiology

Alport syndrome is the second only to ADPKD as most common inherited cause of renal failure. A prevalence of 1 in 5000 in Utah was reported in the late 1980s, but in most populations, the prevalence is much lower than this. Scandinavian studies found an incidence of 1 in 53,000 in Finland [5] and 1 in 17,000 in male births in southern Sweden. Europe-wide, the underlying primary renal disease is reported to be Alport syndrome in approximately 1% of patients reaching ESRF. In paediatric and adolescent populations, this proportion is closer to 2% [6].

Figures from the UK (◘ Fig. 62.3) are consistent with this and show that the proportion has remained stable over almost two decades, whilst the number of patients kept alive by dialysis and transplantation has only recently shown signs of plateauing.

62.2.2 Aetiology and Pathogenesis

The underlying defect in Alport syndrome is a mutation in one of the three genes encoding the α chains of the α345 type IV collagen molecule described above [3]. In the most common form of the disease, X-linked Alport syndrome, the mutation arises in the gene encoding the α5 chain, *COL4A5*, located on the long arm of the X chromosome.

62.2.2.1 X-Linked Alport Syndrome

Mutations in *COL4A5* were first described in the early 1990s [5] and paved the way for the discovery of hundreds of different mutations. A significant proportion of patients develop the disease as a result of de novo mutations. There are no documented hotspots, and most affected families carry unique mutations [7].

Jais (2000) [8] correlated the natural history of X-linked Alport syndrome with the type of underlying gene mutation in a large cohort of male patients. Large deletions, and nonsense mutations and frameshift mutations that led to early chain termination, were associated with a higher probability of reaching end-stage disease and hearing loss by the age of 30 than missense mutations. This has been confirmed by subsequent studies.

In X-linked Alport syndrome, affected men cannot give the disease to their sons, but all of their daughters will carry the affected gene. The offspring of these female carriers, male or female, have a one in two chance of inheriting the mutant gene.

Women who carry one copy of a defective *COL4A5* gene are at increased lifetime risk of developing significant renal disease. A few become severely affected, some

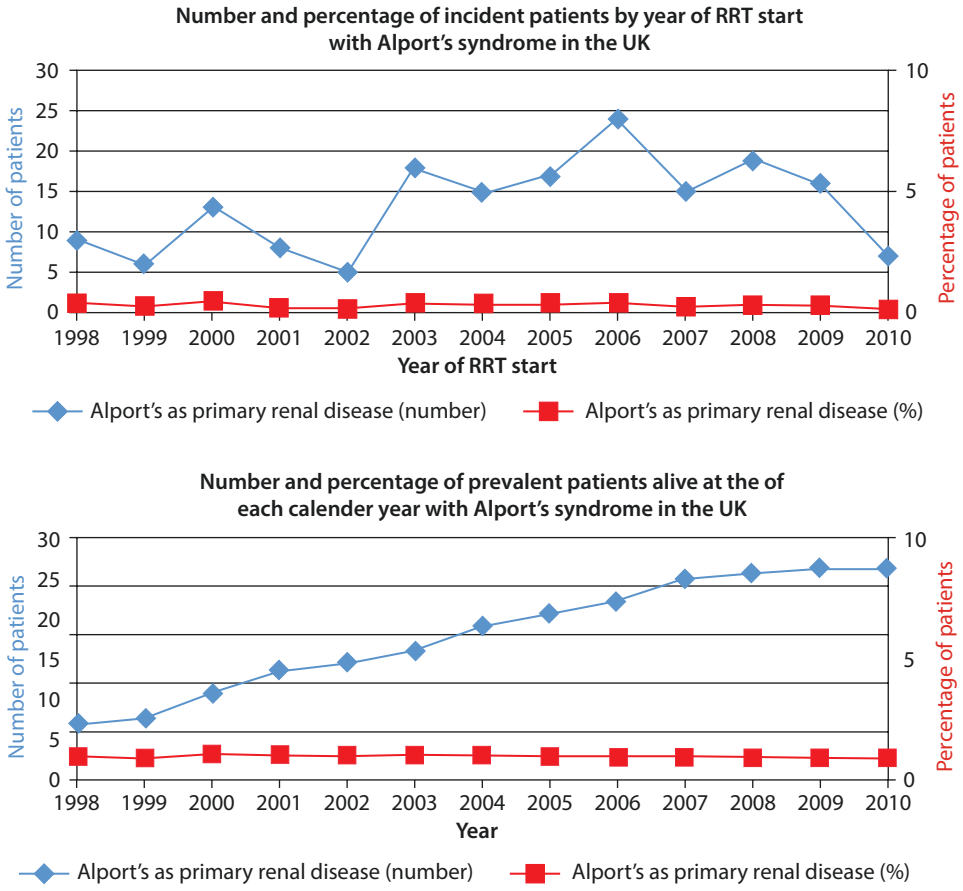

Fig. 62.3 Incidence and prevalence of Alport syndrome in UK RRT population. (Data from UK Renal Registry (► http://www.renalreg.com/))

developing significant manifestations in adolescence or early adulthood. This is discussed further below.

62.2.2.2 Autosomal Recessive Alport Syndrome

In the less common autosomal recessive form of the disease, the mutations occur in the genes encoding for the α3(IV) and α4(IV) chains, *COL4A3* or *COL4A4*, located on chromosome 2.

62.2.2.3 Having Both an Abnormal and a Normal Copy of an Autosomal Alport Gene

Carriers of abnormal autosomal Alport genes are at increased risk of renal disease. Although absolute risk is not characterised, the risk to autosomal carriers of *COL4A3/4* mutations seems probably less than that to female carriers of *COL4A5* mutations.

In some families, ESRF has occurred frequently enough that the label autosomal dominant has been applied. However, the disease in autosomal Alport single mutation carriers is generally much later in onset, usually weakly penetrant within families, and not consistently associated with deafness or other Alport features.

62.2.2.4 Unsuspected Alport Mutations

Through modern, less targeted genetic studies, unexpected Alport mutations are being reported repeatedly in two particular circumstances. The first is in those with familial renal disease without any characteristic features, where whole exome or whole genome sequencing has been carried out. Here, unexpected Alport mutations are a common or even the most frequent explanation (e.g [9].). The second is in nephrotic syndrome in childhood [10].

Reports are also appearing of various second mutations, often in a podocyte protein, that exacerbate the effect of a single *COL4* mutation.

62.2.3 Clinical Features

The genetic heterogeneity of Alport syndrome is reflected in the variable clinical course of the disease. The genetic basis and corresponding clinical features of Alport syndrome are summarised in ◘ Table 62.2. 'Classic' Alport syndrome refers to the X-linked disease in males and the autosomal recessive form in either sex.

Table 62.2 Incidence, affected gene and clinical features of Alport syndrome

Alport syndrome			
Inheritance	**Proportion of affected families**	**Affected gene**	**Clinical picture**
X-linked dominant	85%	*COL4A5*	Severe in male patients, progression to ESRF, high incidence of deafness
			No father-son transmission
			Variable course in females, with most never progressing to ESRF
Autosomal recessive	10–15%	*COL4A3/4*	Severe form of disease in homozygotes, male or female
			Haematuria in father of affected male
			Usually no overt disease in parents of patient
			Variable course in heterozygous carriers, rarely including progression to ESRF
Autosomal (other)	Uncertain	*COL4A3/4*	Male-to-male transmission
			Often no manifestations and when affected generally much milder than in X-linked or recessive disease
			Progression to ESRF (if at all) usually >50 years. No ocular involvement

62.2.4 Renal Disease

Persistent microscopic haematuria is the hallmark of classic Alport syndrome, when it is detectable in the first years of life. Episodes of macroscopic haematuria may occur in infancy or childhood. Dipstick haematuria is also found in 95% of female 'carriers' of X-linked disease [11].

Significant proteinuria typically appears in childhood or adolescence and becomes progressively more severe. It may become very severe. Nephrotic syndrome is increasingly recognised as a presenting feature, so that it is now appropriate to include *COL4* in gene panels for investigation of nephrotic syndrome in childhood [10].

In X-linked disease, affected males almost all eventually develop renal failure. The clinical course in autosomal recessive Alport syndrome for both males and females is essentially the same.

Other autosomal forms of Alport syndrome, including those labelled as autosomal dominant, generally have a more benign course. Progression to end-stage renal disease, if it occurs, is usually over the age of 50 years, and in most families, this is a very infrequent outcome [5, 12]. The co-inherited genes, or environmental factors that account for the increased incidence in some families, are the subject of current research.

62.2.5 Carriers and Heterozygotes

Female carriers of X-linked Alport syndrome had a significant rate of serious renal disease in Jais' 2003 study [7]: 12% progressed to end-stage renal failure by the age of 40 and 30% by the age of 60. These figures are likely to be higher than the true incidence because more severely affected individuals are more likely to be followed up, but most large centres have transplanted or dialysis patients with this background.

The phenotype for heterozygous carriers of autosomal recessive Alport syndrome is generally mild, but not consistently benign. Most are asymptomatic, and fewer seem to have microscopic haematuria. Comprehensive data is not yet available, but end-stage renal failure does occur with increased frequency in such carriers. Why should they have better outcomes than female carriers of X-linked disease? Perhaps the patchy absence of α5 expression (caused by lyonisation, the silencing of one X chromosome) is worse than consistent, evenly distributed under-expression.

Interestingly, there appears to be no correlation between the type of mutation and outcome in female carriers of X-linked disease. Perhaps a 'second hit' in these patients makes more difference than the underlying mutation.

The fact that autosomal mutations can occasionally cause dominantly expressed disease, albeit usually milder than classic Alport syndrome, suggests that there may be a gradation of mutation types and expression, or co-inheritance of other genes that influence outcome, or susceptibility to second hits to cause more severe renal damage. It is known that being a 'carrier' of an autosomal *COL4A3* or *COL4A4* mutation is associated with thin GBM nephropathy, which was historically characterised as a benign condition without long-term threat.

It is wise to be slightly guarded about long-term prognosis for carriers of mutations in autosomal *COL4* genes and definitely guarded about long-term prognosis for female carriers of *COL4A5* mutations.

62.2.6 Hearing Loss

The most common extra-renal manifestation is bilateral sensorineural deafness, the severity of which is variable. High-tone loss is the earliest change. Affected children are not born deaf; hearing loss is progressive during childhood or early adult years. In X-linked disease, 79% of males and 28% of females are said to be affected, although if females get hearing loss, it is usually much later. The degree of hearing impairment is varied too, but never complete, and with hearing aids, good communication is almost always preserved.

Males and females with autosomal Alport syndrome are affected in a similar manner to males with X-linked disease. The pathogenesis of hearing loss in Alport syndrome is not fully understood, but the 345 type IV collagen network is expressed in the cochlea.

62.2.7 Ocular

The most common eye finding is a dot-and-fleck retinopathy, which does not affect visual acuity [13]. Ocular involvement is seen in a third of males with X-linked disease; though as it is progressive, it depends on how severely affected or old the subject is when screened. Less common but much more specific is anterior lenticonus, in which the lens becomes misshapen as a consequence of thinning of the COL4A 345-containing lens capsule. This is typically a late change which occurs after years of renal failure. The retinopathy may be seen earlier, but it is often absent at the time the diagnosis is contemplated. It has been suggested that its appearance in a mutation-bearing parent may be helpful, though this has not been confirmed.

Ocular abnormalities have not been reported in non-recessive autosomal variants of Alport syndrome.

62.2.8 Contiguous Gene Syndromes

Leiomyomatosis is seen in a small proportion of patients with Alport syndrome, as a result of a large deletion involving the proximal (5′) end of the *COL4A5* gene which extends into the proximal part of the adjacent *COL4A6*. The oesophagus, tracheobronchial tree and female genital tract are affected [14]. As the leiomyomatosis component is dominantly expressed, female carriers are fully affected by it even when they have a mild renal phenotype.

AMEE (Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis) is an even more rare contiguous gene deletion syndrome [11].

62.2.9 Differential Diagnosis

The principal differential diagnoses are other haematuric diseases, including TBMN, but also IgA nephropathy or other low-grade nephritis.

A common cause of mislabelling is the expectation of deafness at the time of presentation (it may only become apparent later) and the assumption that deafness with renal failure is always Alport syndrome. Other diseases in which deafness and renal failure occur are listed in Table 62.3.

Table 62.3 Deafness and renal failure

Condition	Features
1. Alport syndrome	Progressive, high-tone deafness that is not present in early childhood *COL4A3-5* genes
2. Branchio-oto-renal syndrome	Renal hypoplasia or malformation. Other manifestations variable and may not be obvious: some or all of deafness, preauricular pits, abnormal pinnae, sinuses in the neck from branchial fistula (or scars from operation). *EYA*, *SIX1*, *SIX5* genes implicated
3. MYH9 mutations	Epstein-Fechtner syndrome; autosomal dominant renal disease – not necessarily glomerular?; with deafness, giant platelets. Epstein has no leucocyte inclusions, nor cataract formation
4. Mitochondrial DNA mutations	Look for other manifestations such as myopathy, diabetes and acidosis
5. Very small print	Other manifestations usually prominent; DIDMOAD syndrome with diabetes mellitus, diabetes insipidus, distal renal tubular acidosis with deafness, etc.
6. Coincidence	Deafness is the most common congenital abnormality

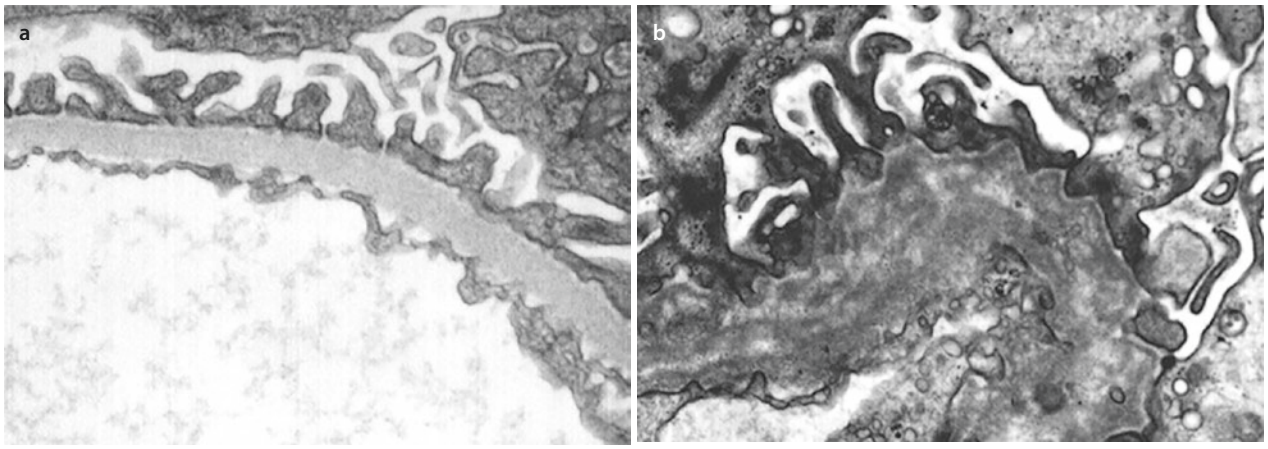

Fig. 62.4 Electron microscopy of the GBM in a normal kidney **a** and in Alport syndrome **b**

62.2.10 Investigations

Early diagnosis of Alport syndrome has become important now that there is evidence for benefit from treatment with ACE inhibitors [15].

Thirty years ago, criteria for diagnosing Alport syndrome for a research study were (1) positive family history, (2) sensorineural hearing loss, (3) ocular changes and (4) typical ultrastructural changes of the GBM [16]. Now however, modern genetic testing has reduced the primacy of both family history and biopsy. If you can demonstrate definitely pathogenic mutation(s) in COL4A3/4/5 in the presence of supportive features, family history and ultrastructure of glomeruli become optional.

Diagnoses in the clinical setting must now also be influenced by new appreciation of the wide range of effects associated with an Alport mutation beyond the classic Alport phenotype. Molecular testing is playing an increasing part.

Formal audiographic testing and ophthalmological referral have been historically recommended as part of diagnostic workup. But they are only reliable in most severe cases, which are often not the ones causing diagnostic difficulty. They are easy to justify if there are symptoms or signs.

62.2.11 Renal Biopsy

Light microscopy of glomerular tissue is mainly useful in ruling out other diagnoses. Changes seen are non-specific and in early disease may include podocyte hypertrophy and capillary wall stiffness. Focal segmental glomerulosclerosis (FSGS) is a common finding and is sometimes erroneously applied as a diagnostic label. Later in the disease process, diffuse glomerular sclerosis is seen.

In early disease, direct immunofluorescence studies are negative. Non-specific deposits of IgM and C3 may be seen in sclerosed glomeruli in more advanced disease [17].

Electron microscopy is required to demonstrate the sometimes dramatic changes in the GBM (Fig. 62.4). The normal architecture is destroyed, replaced by irregularly thickened GBM with multiple splits and lamellae. Not all patients exhibit such impressive ultrastructural changes, and those with earlier disease, or carriers, characteristically show thinning of the GBM. This can be the only abnormality in a significant proportion of adults with the disease, before irregular thickening, advancing to the characteristic and almost pathognomonic 'basket weave' pattern.

Indirect immunofluorescence for the presence of the (IV) collagen α3α4α5 network is possible and can be useful. In an affected patient, it may be negative for all three chains on architecturally preserved glomeruli, whilst positive on control sections. This means obtaining a biopsy early in the disease.

Presence of α3α4α5 chains cannot exclude the diagnosis however, as some mutations cause reduced level of α3α4α5 network rather than absence. Binding of antibodies to α3/4/5(IV) may be segmental in female carriers of X-linked disease, but is normal in carriers of autosomal disease and in patients with TBMN.

62.2.12 Genetic Testing

As newer techniques expand the possibilities and reduce the cost, genetic testing is rapidly becoming an integral part of diagnosing new families in developed countries. Sequencing of all three genes has become the current standard.

Genetic testing is not essential though. In many cases, the diagnosis and inheritance are obvious, and of course, the presence of a mutation does not preclude the presence of other conditions, so a renal biopsy may be

necessary anyway. This perception is likely to shift further as the cost of genetic testing falls, and quality and experience rise, including discovery of genes associated with disease progression.

Genetic testing is most helpful in confirming a probable or likely diagnosis. It is less useful in 'possible' disease, as a negative result does not rule out the diagnosis. It is important to note that:

- Sequencing does not identify mutations in all cases, even when the diagnosis and inheritance pattern are certain. Success rate is however now high.
- As most mutations are unique to a family, the significance of a newly identified mutation may be uncertain. A large deletion or mutation affecting a key amino acid can be labelled as near-certain, but other types of mutation rely on computer prediction, analogy from other cases or in vitro studies. These may make a less certain prediction of relevance.

Once a mutation is identified in a family, it becomes much easier (and less costly) to identify other family members with the same mutation. This can also be done as an antenatal test or before implantation of embryos (preimplantation diagnosis).

Definite identification of carrier status is becoming valuable in screening potential living related donors. Both phenotype and long-term prognoses of carriers are variable (see above), so knowing the genetic status may be helpful in considering the risk of donation.

As more patients are sequenced, knowledge of the significance of gene variants is increasing rapidly. It is important that this knowledge is shared widely.

62.2.13 Skin Biopsy

As the 556 network of type IV collagen is expressed in epidermal basement membrane, it is an attractive idea that COL4A5 mutations (but not autosomal mutations, as α3 and α4 are not expressed in skin) could be detected by skin biopsy. Its use in diagnosis is however limited by technical difficulties and by the variability of α5(IV) expression in affected individuals [18].

Demonstration of α3(IV) or α5(IV) in glomeruli (see above) is more reliable if an early-stage biopsy is available, remembering that presence of antigen does not rule out the disease.

62.3 Treatment

62.3.1 Drug Therapy

Gross et al. published data in 2012 [15] implying substantial long-term benefits from the early use of ACE inhibitors in patients with AS. In this European longitudinal observational study of 283 patients with biopsy-proven or genetically identified AS, it seemed that males with X-linked AS, or homozygotes for autosomal AS, had end-stage renal failure substantially delayed by ACE inhibitors. Previous studies had demonstrated slowed progression in animal models.

Current recommendations [16] are to commence ACE inhibitors or angiotensin receptor blockers in any patient likely to have 'classic' Alport syndrome who has established proteinuria at any increased level, including children. Dose should be titrated to maximum tolerated, which may be less than full dose in young patients. Advice needs to be more nuanced in 'carriers' of abnormal Alport genes, in whom progression is likely to be slower, considering age, severity of proteinuria, whether any GFR has been lost and possibility of pregnancy.

The mechanism of action of ACE inhibitors in Alport syndrome is not fully understood, but may go beyond their well-recognised anti-hypertensive and anti-proteinuric effects. To date, no other therapies have been shown to improve outcome in man, but several add-on alternatives are under active consideration or study, including targeted genetic interventions.

62.3.2 Renal Transplantation

Patients with Alport syndrome benefit from renal transplantation with good long-term outcomes [19]. Recurrence of Alport syndrome in the transplanted kidney does not occur, providing the organ donor does not have the disease. However, two specific issues arise.

62.3.2.1 Living Donation

The use of Alport (usually female) carriers as live kidney donors to their affected relatives seems risky in the light of long-term data on risk to female carriers, but transplants have taken place [6]. Although it seems a less than perfect option, it could be considered in older donors with no proteinuria (in women, usually after child-bearing years) and if both donor and recipient fully understand and accept the increased risk of renal failure.

62.3.2.2 Post-transplant Anti-GBM Nephritis

This rare but devastating complication of renal transplantation in Alport syndrome was first described in 1982 [20], was always rare (probably well below 5%) but now seems to be even more rare. It is a consequence of the recipient generating an immune response to a molecule in the donor kidney that they have not encountered before. It is more likely if the underlying genetic defect is a gene deletion, so that the recipient has seen no protein, rather than a slightly changed one. In line with that, the target is usually the collagen chain that is affected by the mutation. As this is usually α5(IV), the resulting antibodies are likely to be different from the

anti-α3 antibodies of spontaneous, autoimmune anti-GBM (Goodpasture) disease and may not be identified by specific anti-GBM antibody assays [21].

Immunofluorescence of the renal biopsy reveals that antibody fixation to the GBM is relatively common and in most cases is not associated with glomerular damage and does not progress to anti-GBM nephritis. Binding to Bowman's capsule is typically strong if antibodies are anti-α5 chain, whereas this membrane contains less α3(IV) than GBM as the Col(IV) 56 network is found here.

When the disease occurs, it is typically diagnosed months to years after a first transplant, weeks to months after a second and days to weeks after a third. There are haematuria and subsequently often proteinuria, with progressive graft dysfunction that is often initially attributed to rejection. Crescentic nephritis is seen, but lung haemorrhage is not a feature, in contrast to classic anti-GBM disease.

Once identified (usually late), the prognosis for graft survival is very poor, with failure ensuing in near to 90%. The risk of developing the disease again increases with subsequent transplants as described by Browne et al. [21]. In 16 cases of retransplantation, anti-GBM nephritis was seen in 15, with 12 of those grafts damaged irretrievably. Patients seeking retransplantation in this context, and their clinicians, need to be made fully aware of the slim chances of success.

No treatment has been proven to be effective, though sometimes recurrences have been unaccountably less severe and therapy apparently has been more effective. The use of anti-B-cell therapies apart from cyclophosphamide has not been extensively reported, but almost every other option has.

62.3.3 Thin Basement Membrane Nephropathy

Thin basement membrane nephropathy (TBMN) is a disorder in which the GBM is uniformly thinned. The earliest description is likely to date from 1926 [22]. In the past, it was commonly known as 'benign familial haematuria', though we now know that the condition does not always run a benign course.

62.3.4 Epidemiology

This condition is common. Though it is difficult to know the precise prevalence, it has been estimated to be 1%, though post-mortem and transplant data (definitely not synonymous with the general population) have suggested up to 9% [4, 23]. TBMN is the most common inherited renal condition and the most common cause of persistent glomerular haematuria. It is estimated that it is the underlying diagnosis in about a quarter of patients referred to nephrology services for investigation of asymptomatic haematuria [24].

62.3.5 Clinical Features

Microscopic haematuria is the sine qua non of TMN. Episodes of gross haematuria and flank pain also feature in some patients. Proteinuria is not a typical finding in children. Detectable proteinuria may occur in adults, hinting that this is not necessarily a benign condition. However, in the majority of cases, the disease does not lead to significant renal impairment. In a very small number of cases, progressive renal failure has been described without other obvious explanation. In contrast to Alport syndrome and the other rare inherited GBM diseases, extra-renal manifestations are not generally seen in TMN.

62.3.6 Differential Diagnosis

The differential diagnosis of TMN includes any condition that can cause isolated haematuria and therefore can be the early stage of any inflammatory glomerulonephritis. IgA nephropathy is the most likely alternative diagnosis in Europe. Alport syndrome should also be considered, and a family history sought. C3 hereditary nephritis, described in several Cypriot families, should also be considered in the differential of a familial haematuric syndrome [25].

62.3.7 Investigation

Histologically, there is thinning of the basement membrane in the absence of any other morphological changes. Normal GBM thickness is in the range of 350–450 nm. In TMN, this is reduced to less than 250 nm in greater than 50% of the GBM [26]. These findings are similar to those seen in early Alport syndrome, but distinct to the gross distortion of architecture seen later in some patients with Alport syndrome.

A genetic linkage to the *COL4A3* or *COL4A4* locus was identified in 40% of families with TBMN in one research study [27]. Matching mutations have been found in autosomal recessive Alport syndrome. Patients with TBMN who have such mutations can be regarded as carriers of the recessive form of Alport syndrome, with the less certain prognosis described above, and as studies of selected families who carry single copies of

autosomal COL4 mutations sometimes show (above). However, the linkage studies, and unpublished observations, suggest that many cases of TBMN are not linked to Alport syndrome genes, and other explanations must pertain.

Perhaps we need to understand the condition better before genetic testing becomes a standard part of its investigation of TBMD. The balance of utility could change if there was proteinuria or loss of function, providing a stronger possibility of a diagnosis of Alport syndrome. That distinction may otherwise be difficult in the absence of a clear family history.

Immunohistochemical analysis of the distribution of type IV collagen chains has the disadvantages outlined under Alport syndrome.

62.3.8 Management

There is no specific treatment, though in the light of newer uncertainty about long-term prognosis, as outlined above for Alport carriers, long-term monitoring is indicated, e.g. annual blood pressure, urinalysis and occasional creatinine measurement, not necessarily by a nephrologist.

The uncertain long-term outcome of those who do have heterozygous *COL4* mutations, described above, suggests that if there is significant proteinuria, there are good reasons to favour ACE inhibitors [16]. More is likely to be learned in the future about underlying causes and outcomes in those without such mutations or where it is not yet studied. The same mostly upbeat, but slightly guarded, long-term prognosis, and recommendation for continuing occasional monitoring, applies to both.

62.3.9 Nail-Patella Syndrome (Hereditary Osteo-onychodysplasia)

Nail-patella syndrome (NPS) is a rare, autosomal dominant inherited condition in which renal involvement affects only a minority, but sometimes severely. Descriptions of patients with clinical findings similar to those seen in NPS date back to the early nineteenth century. The incidence is thought to be around 1 in 50,000 live births [28].

Nails and patellae exhibit some of the most striking changes, but other common abnormalities are outlined in Table 62.4.

Sweeney et al. looked at a group of 123 patients with NPS [29] and identified end-stage renal disease in only 2%. The prevalence is likely to have risen because of the availability of renal replacement therapy: proteinuria was much more common.

Table 62.4 Clinical features of nail-patella syndrome

Nails	Dysplastic or hypoplastic nails, mainly affecting the thumbs and index fingers
Skeletal	Underdeveloped or absent patellae
	Elbow contracture
	Iliac horns (visible on X-rays)
Renal	Fibrillar collagen bundles within the GBM
Eyes	Open-angle glaucoma

The implicated gene is LMX1B, found on chromosome 9. LMX1B is a transcription factor that influences expression of multiple glomerular genes.

Light microscopy of renal tissue shows subtle and non-specific changes, the most common being focal thickening of the GBM. Electron microscopy is required to reveal the abnormal accumulation of fibrillar type III collagen in the GBM and mesangium. Type III collagen is an interstitial collagen not normally found in the kidneys. Renal disease in NPS is associated with abnormal accumulation of non-glomerular basement membrane components, unlike Alport syndrome where the defect is in a molecule native to the GBM.

62.3.9.1 LMX1B Mutations Without NPS

LMX1B is another gene that is being unexpectedly identified in the absence of recognised extra-renal features in blind sequencing approaches to the investigation of familial renal disease (e.g [30].).

62.3.10 *Laminin* Mutations, Pierson Syndrome and Proteinuria

In 1963, Pierson described siblings with eye abnormalities, congenital nephrotic syndrome and rapid development of end-stage renal failure. Forty years later, the genetic defect was localised to the *LAMB2* gene encoding the β2 chain of laminin [26], part of the dominant laminin 521 isoform that is the major laminin of adult GBM. β2 laminin is also found in other locations that mirror the clinical manifestations. Several ocular abnormalities have been described, with microcoria (fixed constriction of the pupil) being the most characteristic finding.

The original cohort described in the 1960s had severe disease, often fatal in infancy, but it is now appearing that milder mutations may have less florid systemic, predominantly renal phenotypes and indeed potential to exacerbate *COL4* mutations [31]. A role for LAMA5 mutations in proteinuric renal disease is also being suggested [32].

62.3.11 *COL4A1* Mutations and HANAC

Hereditary angiopathy with nephropathy, aneurysms and cramps (HANAC) has been associated with mutations in the COL4A1 gene, encoding the ubiquitous α1-chain of type IV collagen. Because this is such a universal and core basement membrane component, it understandable that only minor mutations are compatible with life.

Plaisier and colleagues [33] described a complex phenotype in which the renal manifestations were a relatively minor component and included haematuria, decreased GFR and renal cysts. Histological and ultrastructural analysis revealed normal appearances of the GBM, but the basement membranes of Bowman's capsule, renal tubules and interstitial capillaries showed irregular thickening and splitting. HANAC is therefore not a disease of the GBM itself, but of other basement membranes within the kidney.

HANAC should be considered in the differential diagnosis of unexplained haematuria in a 'syndromic' patient. Rare mutations associated with renal disease without obvious systemic phenotypes have been identified for this gene too [27].

Chapter Review Questions

1. What are the top 5 causes to explain deafness with renal failure?
2. What simple information do you want that could be very helpful?
3. What is the best diagnostic approach here?
4. What is the relevance of his mother remembering an episode of painless macroscopic haematuria as a baby?
5. What would you expect his urine to show now?
6. What do you think of her blood pressure?
7. What are the key differential diagnoses?
8. Why doesn't she have primary FSGS?
9. What management would you recommend and what advice would you give?
10. What is her long-term prognosis?
11. What could make the risk less (or more) acceptable?

Answers

1. Alport syndrome, branchio-oto-renal syndrome, Fechtner/Epstein syndrome (MYH9 mutation). Coincidence, especially if deafness congenital. Aminoglycosides – (Has anyone ever seen that with current aminoglycosides?)
2. Urine dipstick for clues to nature of renal disease
3. Repeat family history; urine dipstick parents and siblings. If positive for blood, consider renal biopsy (in relative) or genetic testing (in him).
4. This is a characteristic feature of Alport syndrome.
5. Probably still substantial blood and protein
6. It's normal for 17, a little below the 50th centile for a woman of that age.
7. Glomerulonephritis (IgA most likely); thin GBM nephropathy; Alport syndrome
8. Haematuria and not nephrotic
9. ACE inhibitor to max tolerated dose, long-term. Stop in pregnancy. Discuss contraception. Discuss risks of pregnancy whilst proteinuric another time.
10. Regardless of the underlying diagnosis and GFR, a PCR of 160 in your teens gives you a high chance of ESRF in later life. ACEi may reduce that.
11. Tubes tied or post-menopausal (no risk of stress from further pregnancies); no other renal risk factors; urgent need for renal transplant and no other donors

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Online Resources

www.rarerenal.org/clinician-information/alport-syndrome Part of the UK Strategy for Rare Kidney Diseases. Offers advice for clinicians and patients.

www.ncbi.nlm.nih.gov/books/NBK1207 GeneReviews on Alport and Thin BM Nephropathy. (Cliff Kashtan).

www.ncbi.nlm.nih.gov/books/NBK1132 GeneReviews on Nail Patella Syndrome. (Elizabeth Sweeney et al).

www.npsuk.org Patient-run site for Nail Patella Syndrome.

www.ncbi.nlm.nih.gov/books/NBK7046 GeneReviews on COL4A1-related disorders (Emmanuelle Plaisier and Pierre Ronco).