18 Alport Syndrome and Thin Basement Membrane Nephropathy

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Introduction

Several forms of familial glomerular hematuria result from mutations that affect type IV collagen, the major collagenous constituent of glomerular basement membranes (GBM): Alport syndrome (AS), thin basement membrane nephropathy (TBMN) and HANAC syndrome. Persistent hematuria is a cardinal feature of each of these disorders. Mutations in any of three type IV collagen genes, *COL4A3*, *COL4A4* or *COL4A5* can cause AS, which is characterized clinically by progressive deterioration of kidney function with associated hearing and ocular involvement in many affected individuals. A majority of affected individuals demonstrate X-linked inheritance; however, autosomal recessive and autosomal dominant transmission is also observed. TBMN, previously known as "benign familial hematuria," generally is non-progressive and does not have associated extra-renal findings. About 40 % of cases of TBMN exhibit mutations in *COL4A3* or *COL4A4* or linkage to one of these genes. Together, AS and TBMN account for about 30–50 % of children with isolated glomerular hematuria seen in pediatric nephrology clinics [1–5]. Hereditary angiopathy with nephropathy, aneurysms and cramps (HANAC syndrome) arises from mutations in the *COL4A1* gene.

Alport Syndrome

Introduction

The first description of a family with inherited hematuria appeared in 1902 in a report by Guthrie [6]. Subsequent monographs about this family by Hurst in 1923 [7] and Alport in 1927 [8] established that affected individuals in this family, particularly males, developed deafness and uremia. The advent of electron microscopy led to the discovery of unique glomerular basement membrane (GBM) abnormalities in patients with AS [9–11], setting the stage for the histochemical [12–14] and genetic [15, 16] studies that resulted in the identification of type IV collagen genes as the sites of disease-causing mutations. AS occurs in approximately 1:50,000 live births and accounts for 1.3 % and 0.4 % of pediatric and adult end-stage renal disease (ESRD) patients in the United States, respectively [17].

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Etiology and Pathogenesis

Type IV Collagen Proteins, Tissue Distribution, and Genes

Six isoforms of type IV collagen, α1(IV)-α6(IV), are encoded by six genes, *COL4A1-COL4A6*. The type IV collagen genes are arranged in pairs on three chromosomes: *COL4A1-COL4A2* on chromosome 13, *COL4A3-COL4A4* on chromosome 2, and *COL4A5-COL4A6* on the X chromosome. The paired genes are arranged in a 5′-5′ fashion, separated by sequences of varying length containing regulatory elements [18, 19]. All type IV collagen isoforms share several basic structural features: a major collagenous domain of approximately 1,400 residues containing the repetitive triplet sequence glycine (Gly)-X-Y, in which X and Y represent a variety of other amino acids; a *C*-terminal noncollagenous (NC1) domain of approximately 230 residues; and a noncollagenous *N*-terminal sequence of 15–20 residues. The collagenous domains each contain approximately 20 interruptions of the collagenous triplet sequence, while each NC1 domain contains 12 conserved cysteine residues. Type IV collagen chains self associate to form triple helical structures or "trimers." The specificity of chain association is determined by amino acid sequences within the NC1 domains and results in only three trimeric species that are found in nature: $\alpha1_2\alpha2$(IV), α3α4α5(IV) and $\alpha5_2\alpha6$(IV) [20]. Unlike interstitial collagens, which lose their NC1 domains and form fibrillar networks, type IV collagen trimers form open, nonfibrillar networks through NC1-NC1 and N-terminal interactions [21].

$\alpha1_2\alpha2$(IV) trimers are found in all basement membranes, whereas α3α4α5(IV) and $\alpha5_2\alpha6$(IV) trimers have a more restricted distribution. In normal human kidneys, α3α4α5(IV) trimers are found in GBM, Bowman's capsules, and the basement membranes of distal tubules, while $\alpha5_2\alpha6$(IV) trimers are detectable in Bowman's capsules, basement membranes of distal tubules and collecting ducts, but not GBM [22, 23]. $\alpha5_2\alpha6$(IV) trimers are also present in normal epidermal basement membranes as well as some alimentary canal, ocular, and vascular basement membranes. α3α4α5(IV) trimers also occur in several basement membranes of the eye and of the cochlea [24–26].

Mutations in any of the *COL4A3*, *COL4A4*, or *COL4A5* genes will affect the formation and composition of affected basement membranes. If any of the α3(IV), α4(IV), or α5(IV) chains are absent due to severe mutations (deletions, frame shift mutations, premature stop codons), then the other collagen chains are degraded and no α3α4α5(IV) trimers are deposited in basement membranes [27]. In this case, the embryonal $\alpha1_2\alpha2$(IV) network persists. Missense mutations, particularly those that affect the glycine residues involved in triple helix formation, may lead to the formation of abnormally folded trimers that are either degraded or deposited into the basement membrane with formation of an abnormal type IV collagen network. Due to a greater number of disulfide bonds, the α3α4α5(IV) network is more highly cross-linked and is more resistant to proteases than the $\alpha1_2\alpha2$(IV) network [27, 28]. Absence of the α3α4α5(IV) network leads to increased distensibility in the lens capsule when tested in experimental models of AS [29]. The glomerular capillary walls of AS patients may also be mechanically weak and provoke pathologic stretch-related responses in glomerular cells [30].

Genetics

AS occurs in three genetic forms: X-linked (XLAS), autosomal recessive (ARAS) and autosomal dominant (ADAS) (Table 18.1). XLAS, caused by mutations in *COL4A5*, was classically thought to account for approximately 80 % of AS patients while ARAS, caused by mutations in both alleles of *COL4A3* or *COL4A4*, accounted for about 15 % of the AS population. Affected males with XLAS are hemizygotes who carry a single mutant *COL4A5* allele, while affected females are heterozygotes carrying normal and mutant alleles. Individuals with ARAS may be homozygotes, with identical mutations in both alleles of the affected gene or they may be compound heterozygotes, with different mutations in the two alleles [31]. With the advent of next generation sequencing, recent studies are suggesting a higher percentage of patients with AS who

Table 18.1 Familial glomerular hematuria due to type IV collagen mutations

	Genetic locus	Protein product	Renal symptoms	ESRD	GBM ultrastructure	Extrarenal manifestations
Alport syndrome						
X-linked	*COL4A5*	α5(IV)	Hematuria Proteinuria Hypertension	All males, some females	Thinning (early) Lamellation (late)	Deafness Lenticonus Perimacular flecks
Autosomal recessive	*COL4A3*	α3(IV)	Hematuria Proteinuria Hypertension	All males and females	Thinning (early) Lamellation (late)	Deafness Lenticonus Perimacular flecks
	COL4A4 (biallelic)	α4(IV)				
Autosomal dominant	*COL4A3*	α3(IV)	Hematuria Proteinuria Hypertension	Males and females (late)	Thinning (early) Lamellation (late)	Deafness
	COL4A4 (heterozygous)	α4(IV)				
Thin basement Membrane Nephropathy	*COL4A3*	α3(IV)	Hematuria	Rare	Thinning	Rare
	COL4A4 (heterozygous)	α4(IV)				
HANAC syndrome						
Autosomal dominant	*COL4A1*	α1(IV)	Hematuria Cysts CKD	?	Normal	Arterial aneurysms Muscle cramps

CKD chronic kidney disease, *ESRD* end-stage renal disease, *GBM* glomerular basement membrane

demonstrate autosomal dominant inheritance than was previously recognized, up to 31 % in one report [32]. ADAS is caused by heterozygous mutations in *COL4A3* or *COL4A4* [33]. Heterozygous mutations in either *COL4A3* or *COL4A4* have been associated with both ADAS and TBMN; however, it is not clear why some individuals develop a progressive nephropathy while others have a benign clinical course [34].

Over 700 pathogenic mutations have been identified in the *COL4A5* gene in patients and families with XLAS [35]. Mutations can be found along the entire 51 exons of the gene without identified hot spots. About 10–15 % of *COL4A5* mutations occur as spontaneous events, therefore a family history of renal disease is not required for a diagnosis of XLAS. A variety of mutation types have been described: large rearrangements (~20 %), small deletions and insertions (~20 %), missense mutations altering a glycine residue in the collagenous domain of α5(IV) (30 %), other missense mutations (~8 %), nonsense mutations (~5 %) and splice-site mutations (~15 %) [36]. The type of *COL4A5* mutation, or *COL4A5* genotype, has a significant impact on the course of XLAS in affected males [36, 37]. In males with a large deletion, nonsense mutation or a small mutation changing the mRNA reading frame, the risk of developing ESRD before age 30 is 90 %. In contrast, 70 % of patients with a splice-site and 50 % of patients with a missense mutation progress to ESRD before age 30 [36]. In addition, the position of a glycine substitution within the gene may also impact the rate of disease progression as those with 5′ glycine missense mutations demonstrate a more severe phenotype than those with 3′ glycine mutations [37]. In contrast to males with XLAS, a statistical relationship between *COL4A5* genotype and renal phenotype cannot be demonstrated in females with XLAS [38].

Clinical Manifestations

Males with XLAS and ARAS inevitably develop end-stage renal disease (ESRD) at a rate that is influenced by genotype [31, 36]. While most females with XLAS have non-progressive or slowly progressive renal disease, a significant minority demonstrates progression to ESRD. The course of AS is similar in females and males with ARAS [31]. In general, patients with ADAS progress less rapidly than patients with XLAS or ARAS and are less likely to have extra-renal manifestations [39].

Renal Symptoms

Persistent microscopic hematuria (MH) occurs in all males with AS, regardless of genetic type, and is probably present from early in infancy. Approximately 95 % of heterozygous females with XLAS have persistent or intermittent MH [38], and 100 % of females with ARAS have persistent MH. Gross hematuria is not unusual in affected boys and girls with Alport syndrome, occurring at least once in approximately 60 % of affected males [36, 40].

In males with XLAS, and in males and females with ARAS, proteinuria typically becomes detectable in late childhood or early adolescence and is progressive. Affected children first demonstrate microalbuminuria that progresses to overt proteinuria with time [41]. In one large cohort of females with XLAS, 75 % were found to have proteinuria, although the timing of onset was not investigated [38].

Blood pressure is typically normal in childhood but, like proteinuria, hypertension is common in adolescent males with XLAS or ARAS, and in females with ARAS. Most females with XLAS have normal blood pressure, but hypertension may develop, particularly in those with proteinuria.

All males with XLAS eventually require renal replacement therapy, with 50 % of untreated males reaching ESRD by age 25, 80 % by age 40 and 100 % by age 60 [36]. The timing of ESRD in patients with ARAS is probably similar to XLAS males, although ARAS patients with normal renal function in their 30s and 40s have been reported [31]. In patients with ADAS, the age at which 50 % of patients have progressed to ESRD is approximately 50 years, or twice as long as XLAS males [39].

Females who are heterozygous for *COL4A5* mutations ("carriers" of XLAS) demonstrate widely variable disease outcomes with some women demonstrating only lifelong asymptomatic hematuria while others develop chronic progressive kidney disease including ESRD [42]. The risk of ESRD is lower in XLAS females than in XLAS males, but it is by no means trivial. About 12 % of XLAS females reach ESRD by age 45, 30 % by age 60 and 40 % by age 80 [38]. The explanation for the wide variability in outcomes for XLAS females is uncertain, but likely multifactorial. Risk factors for ESRD in XLAS females include proteinuria and sensorineural deafness [38, 43]. X-inactivation, the process by which one X chromosome in females is silenced to adjust for gene dosage differences between males and females, may play a role in renal disease progression in XLAS females [44, 45]. In a mouse model of female XLAS, modest skewing of X-inactivation to favor expression of the wild type α5(IV) was associated with a survival advantage [46]. Further studies are required to determine how to accurately predict the risk of progressive renal disease in women who are affected with XLAS.

The Alport nephropathy progresses predictably through a series of clinical phases. Phase I typically lasts from birth until late childhood or early adolescence, and is characterized by isolated hematuria, with normal protein excretion and renal function. In Phase II, initially microalbuminuria followed by overt proteinuria is superimposed on hematuria, but renal function remains normal. Patients in Phase III exhibit declining renal function in addition to hematuria and proteinuria, and those in Phase IV have end-stage renal disease. These phases have histological correlates, as described in the next section. The rate of passage through these phases is primarily a function of the causative mutation, at least in males with XLAS. Patients with *COL4A5* mutations that prevent production of any functional protein (deletions, nonsense mutations) proceed through these phases more rapidly than those whose mutations allow synthesis of a functional,

albeit abnormal, protein (some missense mutations). Females with XLAS can be viewed as passing through the same phases as males, although the rate of progression is typically so slow that the journey to ESRD may not be completed during the individual's lifetime.

Hearing

Newborn hearing screening is normal in males with XLAS, and in males and females with ARAS, but bilateral impairment of perception of high frequency sounds frequently becomes detectable in late childhood. The hearing deficit is progressive, and extends into the range of conversational speech with advancing age. The deficit usually does not exceed 60–70 dB and speech discrimination is preserved, so that affected individuals benefit from hearing aids. Sensorineural hearing loss (SNHL) is present in 50 % of XLAS males by approximately age 15, 75 % by age 25, and 90 % by age 40 [36]. Like the effect on renal disease progression, missense mutations in COL4A5 are associated with an attenuated risk of hearing loss. The risk of SNHL before age 30 is 60 % in patients with misssense mutations, while the risk of SNHL before age 30 is 90 % in those with other types of mutations [36]. SNHL is less frequent in females with XLAS. About 10 % of XLAS females have SNHL by 40 years of age, and about 20 % by age 60 [38]. SNHL is common in ARAS as well with approximately 66 % of individuals affected [31].

The SNHL in AS has been localized to the cochlea [47]. In control cochleae, the α3(IV), α4(IV) and α5(IV) chains are expressed in the spiral limbus, the spiral ligament, stria vascularis and in the basement membrane situated between the Organ of Corti and the basilar membrane [48–50]. However, these chains are not expressed in the cochleae of ARAS mice [49], XLAS dogs [50] or men with XLAS [26]. Examination of well-preserved cochleae from men with XLAS revealed a unique zone of separation between the organ of Corti and the underlying basilar membrane, as well as cellular infiltration of the tunnel of Corti and the spaces of Nuel [51]. These changes may be associated with abnormal tuning of basilar membrane motion and hair cell stimulation, resulting in defective hearing. An alternative hypothesis is that hearing is impaired by changes in potassium concentration in the scala media induced by abnormalities of type IV collagen in the stria vascularis [52].

Ocular Anomalies

Abnormalities of the lens and the retina are common in individuals with AS, typically becoming apparent in the second to third decade of life in XLAS males and in both males and females with ARAS. The α3(IV), α4(IV) and α5(IV) chains are normal components of the anterior lens capsule and other ocular basement membranes, and mutations that interfere with the formation or deposition of α3α4α5(IV) trimers prevent expression of these chains in the eye [24, 48]. Anterior lenticonus, which is considered virtually pathognomonic for AS [53], is absent at birth and manifests during the second and third decades of life in ~13–25 % of affected individuals [36, 54]. In this disorder, the anterior lens capsule is markedly attenuated, especially over the central region of the lens, and exhibits focal areas of dehiscence leading to refractive errors and, in some cases, cataracts [55, 56]. Anterior lenticonus has been described only rarely in heterozygous females with *COL4A5* mutations [38]. Dot-fleck retinopathy, a characteristic alteration of retinal pigmentation concentrated in the perimacular region [57], is also common in AS patients and does not appear to be associated with any abnormality in vision [36]. Recurrent corneal erosions [58, 59] and posterior polymorphous dystrophy, manifested by clear vesicles on the posterior surface of the cornea [60], have also been described in AS.

Leiomyomatosis

Several dozen families in which AS is transmitted in association with leiomyomas of the esophagus and tracheobronchial tree have been described [61]. Affected individuals carry X-chromosomal deletions that involve the *COL4A5* gene and terminate within the second intron of the adjacent *COL4A6* gene [62–64]. Those affected tend to become symptomatic in late childhood, and may exhibit dysphagia, postprandial vomiting, epigastric or retrosternal pain,

recurrent bronchitis, dyspnea, cough or stridor. Females with the AS-leiomyomatosis complex typically have genital leiomyomas, with clitoral hypertrophy and variable involvement of the labia majora and uterus.

Other Findings

AS associated with mental retardation, mid-face hypoplasia and elliptocytosis has been described in association with large *COL4A5* deletions that extend beyond the 5′ terminus of the gene [65]. Early development of aortic root dilatation and aneurysms of the thoracic and abdominal aorta, as well as other arterial vessels, have been described in AS males, perhaps due to abnormalities in the $\alpha5_2\alpha6$(IV) network in arterial smooth muscle basement membranes [66].

Renal Histopathology

Children with AS typically show little in the way of renal parenchymal changes by light microscopy before about 5 years of age. In older patients, mesangial hypercellularity and matrix expansion may be observed. As the disease progresses, focal segmental glomerulosclerosis, tubular atrophy and interstitial fibrosis become the predominant light microscopic abnormalities. Although some patients exhibit increased numbers of immature glomeruli or interstitial foam cells, these changes are not specific for AS.

Electron microscopy of renal biopsy specimens is frequently diagnostic, although the expression of the pathognomonic lesion is age-dependent and, for those with XLAS, gender-dependent. In early childhood, the predominant ultrastructural lesion in males is diffuse attenuation of the GBM. This may be identical in appearance to patients with TBMN. The classic ultrastructural lesion is diffuse thickening of the glomerular capillary wall, accompanied by "basket-weave" transformation of the lamina densa, intramembranous vesicles, scalloping of the epithelial surface of the GBM and effacement of podocyte foot-processes (Fig. 18.1). These changes are more prevalent in affected males, typically becoming prominent in late childhood and adolescence. Affected females can display a spectrum of lesions, demonstrating either predominantly normal-appearing GBM, focal GBM attenuation, diffuse GBM attenuation, thickening/basket-weaving, or diffuse basket-weaving. The extent of the GBM lesion progresses inexorably in males, although the rate of progression may be influenced by *COL4A5* genotype. Females may have static or progressive GBM lesions. X-chromosome inactivation pattern, age and *COL4A5* genotype could all contribute to the dynamics of GBM change in affected females.

The classic GBM lesion is not found in all kindreds with AS. Adult patients who demonstrate only GBM thinning, yet have *COL4A5* mutations, have been described. Although these represent a minority of Alport patients and families, they highlight the somewhat vague histological distinction between AS and TBMN. This issue is discussed further in the section on TBMN.

Routine immunofluorescence microscopy is normal, or shows nonspecific deposition of immunoproteins, in patients with AS. In contrast, specific immunostaining for type IV collagen α chains is frequently diagnostic, and can distinguish the X-linked and autosomal recessive forms of the disease (Fig. 18.1). The utility of this approach derives from the fact that most disease-causing mutations in AS alter the expression of the $\alpha3\alpha4\alpha5$(IV) and $\alpha5_2\alpha6$(IV) trimers in renal basement membranes. Most *COL4A5* mutations prevent expression of both trimer forms in the kidney, so that in about 80% of XLAS males immunostaining of renal biopsy specimens for α3(IV), α4(IV) and α5(IV) chains is completely negative [67]. About 60–70% of XLAS females exhibit mosaic expression of these chains, while in the remainder immunostaining for these chains is normal. The biallelic mutations in *COL4A3* and *COL4A4* that cause ARAS often prevent expression of $\alpha3\alpha4\alpha5$(IV) trimers but have no effect on expression of $\alpha5_2\alpha6$(IV) trimers. In renal biopsy specimens from patients with ARAS, immunostaining for α3(IV) and α4(IV) chains is negative in the GBM. However, while immunostaining of GBM for the α5(IV) chain is negative due to the absence of $\alpha3\alpha4\alpha5$(IV) trimers, Bowman's capsules, distal tubular basement membranes and

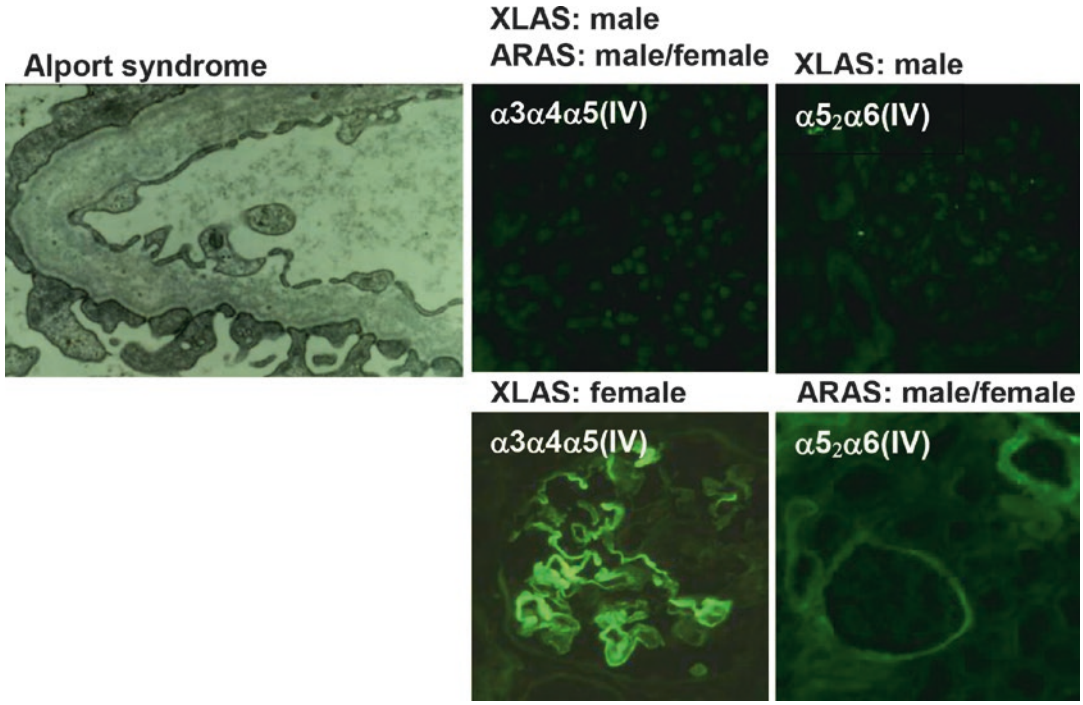

Fig. 18.1 Typical findings on electron microscopy and type IV collagen immunostaining in Alport syndrome. Abbreviations – *XLAS* X-linked Alport syndrome, *ARAS* autosomal recessive Alport syndrome

collecting duct basement membranes are positive for α5(IV), due to the unimpaired expression of $\alpha5_2\alpha6$(IV) trimers. Heterozygous carriers of a single *COL4A3* or *COL4A4* mutation have exhibited normal renal basement membrane immunostaining for α3(IV), α4(IV) and α5(IV) chains when studied.

The $\alpha5_2\alpha6$(IV) trimer is a normal component of epidermal basement membranes (EBM). Consequently, about 80 % of males with XLAS can be diagnosed by skin biopsy on the basis of absence of α5(IV) expression in EBM. In 60–70 % of XLAS females there is a mosaic pattern of immunostaining for α5(IV). EBM expression of α5(IV) is normal in patients with ARAS and in subjects with heterozygous mutations in *COL4A3* or *COL4A4*.

Diagnosis and Differential Diagnosis

AS is just one potential cause of familial and sporadic glomerular hematuria. Accurate diagnosis rests on careful clinical evaluation, a precise family history, selective application of invasive diagnostic techniques and, in appropriate patients, molecular diagnosis.

The presence of isolated microscopic hematuria in a child with a positive family history for hematuria, an autosomal dominant pattern of inheritance, and a negative family history for ESRD strongly suggests a diagnosis of TBMN. Less common conditions associated with familial glomerular hematuria include the autosomal dominant *MYH9* disorders (Epstein and Fechtner syndromes), in which macrothrombocytopenia is a constant feature; familial IgA nephropathy and X-linked membranoproliferative glomerulonephritis.

When family history for hematuria is negative, the differential diagnosis of isolated glomerular hematuria, or hematuria associated with proteinuria, includes IgA nephropathy, the various forms of C3 nephropathy, membranous nephropathy, lupus nephritis, postinfectious glomerulonephritis and Henoch-Schönlein nephritis,

among others, in addition to AS and TBMN. Some of these conditions will be strongly suspected on the basis of clinical findings (e.g., rash and joint complaints) while others will be suggested by laboratory findings, such as hypocomplementemia.

Formal audiometric and ophthalmological examinations should be considered as part of the diagnostic evaluation in children with persistent microscopic hematuria. Audiometry may be very helpful in children over age 6–8 years, especially boys, since high-frequency SNHL would point toward a diagnosis of AS. The presence of anterior lenticonus or the dot-fleck retinopathy may be diagnostic. However, these lesions are more prevalent in patients with advanced disease, and less likely to be present in the young patients in whom diagnostic ambiguity tends to be the greatest.

Tissue studies are appropriate when clinical and pedigree information does not allow a diagnosis of thin basement membrane nephropathy and when AS cannot be ruled out by symptoms and laboratory findings. Several options are available for confirming a diagnosis of AS including skin biopsy, kidney biopsy and mutation analysis. Skin biopsy is often utilized as the initial invasive diagnostic procedure in patients suspected of AS as it is less invasive and expensive than a renal biopsy. On skin biopsy, the majority of subjects with XLAS will display abnormal expression of the α5(IV) chain in epidermal basement membranes (EBM), as described above. Normal EBM α5(IV) expression in a patient with hematuria has several possible explanations: (1) the patient has XLAS, but his or her *COL4A5* mutation allows EBM expression of α5(IV); (2) the patient has ARAS, or ADAS, in which α5(IV) expression is expected to be preserved; or (3) the patient has a disease other than AS. Renal biopsy would then provide the opportunity to diagnose other diseases, to examine type IV collagen α chain expression in renal basement membranes, and to evaluate GBM at the ultrastructural level.

Mutation analysis using conventional Sanger sequencing is capable of identifying *COL4A5* mutations in 80–90 % of males with XLAS [68]. High mutations detection rates in *COL4A3* and *COL4A4* in patients with ARAS are also possible, particularly if there is parental consanguinity. Commercially available genetic testing for mutations in *COL4A3*, *COL4A4*, and *COL4A5* is available in the United States and around the world. Next generation sequencing, which allows simultaneous analysis of *COL4A3*, *COL4A4* and *COL4A5*, appears likely to eventually supplant Sanger sequencing as the preferred approach.

Treatment

The goal of treatment in AS is to slow the progression of kidney disease and delay the need for renal replacement therapy. Several therapeutic approaches have demonstrated efficacy in murine ARAS, including angiotensin blockade [69–71], inhibition of TGFβ-1 [72], chemokine receptor 1 blockade [73], administration of bone morphogenic protein-7 [74], suppression of matrix metalloproteinases [28] and bone marrow transplantation [75]. Cyclosporine therapy slowed progression of kidney disease in a canine model of AS; however, human studies have demonstrated significant nephrotoxicity and adverse effects and this treatment is not recommended [76–78]. Angiotensin converting enzyme (ACE) inhibition also prolonged survival in a canine XLAS model [79]. Uncontrolled studies in human AS subjects have shown that ACE inhibition can reduce proteinuria, at least transiently [80, 81]. A multicenter, randomized, double-blind study comparing losartan with placebo or amlodipine in 30 children with AS demonstrated a significant reduction in proteinuria in the losartan treated group [82]. An extension of this study showed comparable efficacy of either enalapril or losartan in reducing proteinuria in children with AS [83]. A report from the European Alport Registry, which includes 283 patients over 20 years, compared renal outcomes in AS patients treated with ACE inhibition at various time points: at onset of microalbuminuria, at onset of proteinuria, or in CKD stage III-IV [84]. This retrospective review demonstrated a delay in renal replacement therapy by 3 years in the treated CKD group and by 18 years in the treated proteinuric group [84]. Side effects of ACE inhibition were rare and included hyperkalemia (<2 %),

cough (<1 %), and hypotension (<1 %). Based on these findings a prospective, double-blind, randomized, placebo controlled trial is underway to compare outcomes in AS patients treated with ramipril vs. placebo at an early time point (microalbuminuria or isolated hematuria) [85].

Current clinical practice guidelines recommend treatment with an ACE inhibitor for affected individuals with proteinuria, including heterozygous females (Table 18.2) [41]. Treatment should be considered for those with microalbuminuria and a family history of ESRD <30 years of age or a severe *COL4A5* mutation (deletion, splice site, or nonsense mutation) [41, 86]. There are insufficient data to recommend treatment for individuals with microscopic hematuria only; however, the ongoing clinical trial in this population hopefully will shed light on the utility of treatment at this early stage of disease. Treatment of hypertension and other manifestations of advancing disease is of course an important component of therapy for AS. Similar to other children with chronic kidney disease, blood pressures should be controlled to the 50 % for age, gender, and height in children with AS in order to slow the progression of kidney disease [87].

Renal Transplantation

In general, outcomes following renal transplantation in patients with AS are excellent [88]. Clinicians involved in transplantation of AS patients must address two important aspects of the disease. First, the donor selection process must avoid nephrectomy in relatives at risk for end-stage renal disease. Second, post-transplant management should provide surveillance for post-transplant anti-GBM nephritis, a complication unique to AS.

Informed donor evaluation requires familiarity with the genetics of AS and the signs and symptoms of the disease. In families with XLAS, 100 % of affected males and ~95 % of affected females exhibit hematuria. Consequently, males who do not have hematuria are not affected, and a female without hematuria has only about a 5 % risk of being affected. Given an estimated 30 % risk of end-stage renal disease in women with AS [38], these women should generally be discouraged from kidney donation, even if hematuria is their only symptom. A report from Germany described five women with XLAS and one ARAS carrier who served as kidney donors [89]. One donor had proteinuria prior to transplant and all had microscopic hematuria. Three donors developed new onset hypertension and two developed new proteinuria while renal function declined by 25–60 % over 2–14 years after donation in four of the donors, highlighting the increased donor risk in this population [89].

Overt anti-GBM nephritis occurs in 3–5 % of transplanted AS males [90]. Onset is typically within the first post-transplant year, and the disease usually results in irreversible graft failure within weeks to months of diagnosis. The risk of

Table 18.2 Recommendations for intervention based on urinary findings and anticipated disease course

	Family history of early ESRD (<30 years) or severe[a] *COL4A5* mutation		Family history of late ESRD (>30 years) or less severe[b] *COL4A5* mutation	
	Males	Females	Males	Females
Hematuria	Intervention prior to onset of microalbuminuria is not recommended at this time	No	No	No
Hematuria + microalbuminuria	Consider intervention	Consider intervention	No	No
Hematuria + proteinuria	Yes	Yes	Yes	Yes

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ESRD end stage renal disease
[a]Deletion, nonsense, or splice site mutation
[b]Missense mutation

recurrence in subsequent allografts is high. In males with XLAS, the primary target of anti-GBM antibodies is the α5(IV) chain [91, 92]. Both males and females with ARAS can develop post-transplant anti-GBM nephritis, and in these cases the primary antibody target is the α3(IV) chain [91, 93]. The α3(IV) chain is also the target of Goodpasture autoantibodies, but the epitope identified by these antibodies differs from the α3(IV) epitope recognized by ARAS anti-GBM alloantibodies [94].

Thin Basement Membrane Nephropathy

Introduction

The term "benign familial hematuria" (BFH) was historically used to describe kindreds displaying autosomal dominant transmission of isolated, nonprogressive glomerular hematuria [95–97]. Renal biopsy findings in these families are typically limited to GBM attenuation by electron microscopy. In 1996, Lemmink and colleagues were the first to report a heterozygous *COL4A3* mutation in a family with BFH [34]. "Thin basement membrane nephropathy" (TBMN) has gradually become the preferred term for hematuria associated with GBM attenuation and a nonprogressive course (Table 18.1). TBMN generally has a good prognosis; however, there is an increased risk of hypertension, proteinuria, and renal impairment in affected individuals [98]. The prevalence of TBMN is estimated at 1–2 % of the population [99].

In discussing TBMN, it is important to recall that GBM thinning is a pathological description rather than a distinct, homogeneous entity. GBM attenuation can result from hemizygous or heterozygous mutations in *COL4A5* (XLAS), biallelic mutations in *COL4A3* or *COL4A4* (ARAS), heterozygous mutations in *COL4A3* or *COL4A4* (the carrier state for ARAS) or mutations at other unknown genetic loci.

It is the underlying cause of GBM attenuation that determines prognosis, perhaps in combination with remote modifier loci, rather than the GBM thinning itself. Hemizygous mutations in *COL4A5*, and biallelic mutations in *COL4A3* or *COL4A4*, lead to progressive GBM thickening and renal failure, while heterozygous mutations in *COL4A3* or *COL4A4* may be associated with persistent GBM attenuation and a benign outcome (TBMN) or slowly progressive disease (ADAS). Women with heterozygous mutations in *COL4A5* are arrayed across the middle of the prognostic spectrum. The range of outcomes likely reflects differences between cellular responses to complete absence of α3α4α5 trimers (ARAS and hemizygous XLAS), mixed α3α4α5-positive and α3α4α5-negative GBM (heterozygous XLAS) and homogeneous reduction in α3α4α5 content (heterozygous *COL4A3* or *COL4A4* mutations).

Etiology and Pathogenesis

The essential features of the type IV collagen protein family are discussed in the preceding section on AS. It is possible that heterozygous mutations in *COL4A3* or *COL4A4* result in reduction of α3α4α5(IV) trimers in GBM; however, this has not been assessed using quantitative methodologies. Identification of mutations in *COL4A3* or *COL4A4*, or demonstration of genetic linkage to these loci, has been achieved in about 40 % of TBMN families. About 20 mutations in *COL4A3* and *COL4A4*, predominantly single nucleotide substitutions, have been described in TBMN families [5]. Other loci for TBMN have yet to be identified.

It is assumed that the attenuated GBM of TBMN and early AS is mechanically fragile and that, as a result, the glomerular capillary wall has an increased potential for rupture at physiologic levels of intracapillary pressure. This mechanism remains theoretical, since it has never been tested in vivo or in the laboratory. There is indirect evidence in support of this hypothesis. Persistent microscopic hematuria is more common in women, who have relatively thin GBM [100]. Macroscopic hematuria, intermittent or persistent, is fairly common in children with Alport syndrome, but tends to disappear with age, perhaps because the GBM thickens and becomes less susceptible to rupture [40].

Clinical Manifestations

TBMN is the most common cause of persistent microscopic hematuria in children and adults [101]. Children with TBMN typically exhibit persistent microscopic hematuria, although intermittent microhematuria may be observed. Episodic gross hematuria may occur in association with acute infection. Proteinuria is rare in childhood but can be observed in a significant proportion of adult patients [102]. Chronic kidney disease (CKD) or ESRD is observed in <5% of affected adults [102–105]. Extrarenal abnormalities, such as hearing loss or ocular defects, are unusual and probably unrelated.

Histopathology

Light and routine immunofluorescence microscopy typically shows no abnormalities, especially in children. Adult TBMN patients with renal dysfunction or hypertension may exhibit premature glomerular obsolescence [104]. Type IV collagen staining demonstrates no abnormalities in the renal basement membrane expression of the α3-α6(IV) chains, in contrast to patients with AS [14, 106]. Characteristic thinning of the GBM can be identified on electron microscopy. Patients with TBMD typically exhibit diffuse thinning of the lamina densa and, perhaps as a result, of the GBM as a whole. The thickness of normal GBM is age- and gender-dependent. Both the lamina densa and the GBM increase rapidly in thickness between birth and age 2 years, followed by gradual thickening throughout childhood and adolescence [107]. GBM thickness of adult men exceeds that of adult women [108]. Because a variety of techniques have been used to measure GBM width, there is no standard definition of "thin" GBM (Fig. 18.2). The cut-off value in adults ranges from 250 to 330 nm, depending upon technique [109, 110]. For children, the cut-off is in the range of 200–250 nm (250 nm is within 2SD of the mean at age 11) [2, 3, 111].

Diagnosis and Differential Diagnosis

IgA nephropathy, TBMN and Alport syndrome comprise the most common causes of glomerular hematuria in the pediatric population. Careful clinical evaluation and thorough pedigree analysis can help segregate children with glomerular hematuria into those who require renal biopsy or other tissue studies, and those who can be followed prospectively without the need for tissue studies. Since adults with familial hematuria may not be aware that they are affected [112], obtaining urinalyses on the parents of children with hematuria may be very helpful.

In a child with isolated microscopic hematuria, a strong family history of dominantly-transmitted hematuria, and a negative family history for renal failure, a clinical diagnosis of TBMN can be made, and renal biopsy withheld. These children should be monitored every 1–2 years for the development of proteinuria or hypertension, and to update the family history [86].

In the child with GBM attenuation and a negative or limited family history, the challenge for the clinician is to distinguish TBMN and AS. Audiometry and ophthalmologic examination may be helpful if abnormal, but the younger the child, the less useful these tests are, given the usual natural history of hearing loss and ocular changes in AS (see preceding section). Renal biopsy with immunostaining for type IV collagen α3(IV), α4(IV) and α5(IV) chains can be particularly helpful in these situations, as discussed in the section on AS. While molecular analysis of type IV collagen genes is available in a number of research and commercial laboratories, it is often unnecessary to obtain genetic confirmation in patients with TBMN unless the course is atypical. Genetic testing for mutations in *COL4A3-COL4A5* should be considered in individuals with proteinuria, renal impairment, or when AS cannot be excluded based on family history.

Treatment

Treatment is not necessary for the great majority of TBMN patients, especially children, since the course of the disorder is typically benign. Adult patients with proteinuria are theoretically candidates for angiotensin blockade, although there are no specific studies in this area.

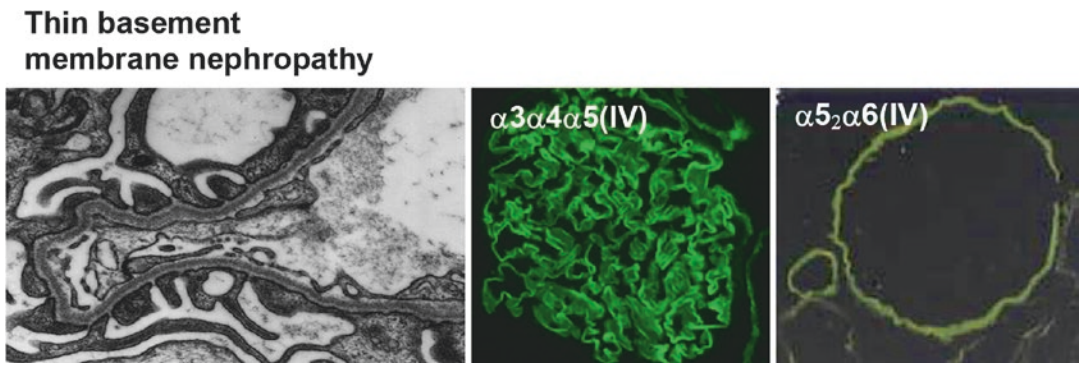

Fig. 18.2 Typical findings on electron microscopy and type IV collagen immunostaining in thin basement membrane nephropathy

Hereditary Angiopathy with Nephropathy, Aneurysms, and Cramps (Hanac Syndrome)

Introduction

This autosomal dominant disorder results from mutations in the *COL4A1* gene (Table 18.1) [113–115]. Complete absence of *COL4A1* is embryonic lethal in mice [116]. Missense mutations that allow for expression of an abnormal α1(IV) chain lead to the development of HANAC syndrome. Renal findings include gross and microscopic hematuria, cysts and chronic kidney disease. Vascular anomalies include cerebral artery aneurysms and retinal arteriolar tortuosity. Affected individuals may have recurrent muscle cramps and elevated creatine kinase levels.

Pathology

No abnormalities of GBM ultrastructure or basement membrane expression of type IV collagen chains have been observed in renal biopsy specimens from affected individuals with hematuria. Irregular thickening, lamellation and focal interruptions of Bowman's capsules, tubular basement membranes and interstitial capillary basement membranes have been described, as well as abnormalities of epidermal basement membranes and dermal arterial basement membranes.

Genetics

The reported mutations in HANAC syndrome families affect highly conserved glycine residues in the collagenous domain of the α1(IV) chain, potentially affecting integrin binding sites.

References

1. Trachtman H, Weiss R, Bennett B, Griefer I. Isolated hematuria in children: indications for a renal biopsy. Kidney Int. 1984;25:94–9.
2. Schroder CH, Bontemps CM, Assmann KJM, Schuurmans-Stekhoven JH, Foidart JM, Monnens LAH, et al. Renal biopsy and family studies in 65 children with isolated hematuria. Acta Paediatr Scand. 1990;79:630–6.
3. Lang S, Stevenson B, Risdon RA. Thin basement membrane nephropathy as a cause of recurrent haematuria in childhood. Histopathology. 1990;16:331–7.
4. Piqueras AI, White RH, Raafat F, Moghal N, Milford DV. Renal biopsy diagnosis in children presenting with hematuria. Pediatr Nephrol. 1998;12:386–91.
5. Rana K, Wang YY, Buzza M, Tonna S, Zhang KW, Lin T, et al. The genetics of thin basement membrane nephropathy. Semin Nephrol. 2005;25:163–70.
6. Guthrie LG. "Idiopathic", or congenital, hereditary and familial hematuria. Lancet. 1902;1:1243–6.
7. Hurst AF. Hereditary familial congenital haemorrhagic nephritis occurring in sixteen individuals in three generations. Guy's Hosp Rec. 1923;3:368–70.
8. Alport AC. Hereditary familial congenital haemorrhagic nephritis. Br Med J. 1927;1:504–6.
9. Hinglais N, Grunfeld J-P, Bois LE. Characteristic ultrastructural lesion of the glomerular basement

membrane in progressive hereditary nephritis (Alport's syndrome). Lab Invest. 1972;27:473–87.
10. Spear GS, Slusser RJ. Alport's syndrome: emphasizing electron microscopic studies of the glomerulus. Am J Pathol. 1972;69:213–22.
11. Churg J, Sherman RL. Pathologic characteristics of hereditary nephritis. Arch Pathol. 1973;95:374–9.
12. Olson DL, Anand SK, Landing BH, Heuser E, Grushkin CM, Lieberman E. Diagnosis of hereditary nephritis by failure of glomeruli to bind antiglomerular basement membrane antibodies. J Pediatr. 1980;96:697–9.
13. McCoy RC, Johnson HK, Stone WJ, Wilson CB. Absence of nephritogenic GBM antigen(s) in some patients with hereditary nephritis. Kidney Int. 1982;21:642–52.
14. Kashtan C, Fish AJ, Kleppel M, Yoshioka K, Michael AF. Nephritogenic antigen determinants in epidermal and renal basement membranes of kindreds with Alport-type familial nephritis. J Clin Invest. 1986;78:1035–44.
15. Atkin CL, Hasstedt SJ, Menlove L, Cannon L, Kirschner N, Schwartz C, et al. Mapping of Alport syndrome to the long arm of the X chromosome. Am J Hum Genet. 1988;42:249–55.
16. Barker DF, Hostikka SL, Zhou J, Chow LT, Oliphant AR, Gerken SC, et al. Identification of mutations in the COL4A5 collagen gene in Alport syndrome. Science. 1990;248:1224–7.
17. USRDS. USRDS 2013 Annual data report: Altas of Chronic Kidney Disease and End-Stage Renal Disease in the United States. Bethesda, National Institutes of Health NIoDaDaKD; 2013.
18. Poschl E, Pollner R, Kuhn K. The genes for the alpha 1(IV) and alpha 2(IV) chains of human basement membrane collagen type IV are arranged head-to-head and separated by a bidirectional promoter of unique structure. EMBO J. 1988;7(9):2687–95.
19. Segal Y, Zhuang L, Rondeau E, Sraer JD, Zhou J. Regulation of the paired type IV collagen genes COL4A5 and COL4A6. Role of the proximal promoter region. J Biol Chem. 2001;276(15):11791–7.
20. Khoshnoodi J, Cartailler JP, Alvares K, Veis A, Hudson BG. Molecular recognition in the assembly of collagens: terminal noncollagenous domains are key recognition modules in the formation of triple helical protomers. J Biol Chem. 2006;281(50): 38117–21.
21. Hudson BG. The molecular basis of Goodpasture and Alport syndromes: beacons for the discovery of the collagen IV family. J Am Soc Nephrol. 2004;15(10):2514–27.
22. Yoshioka K, Hino S, Takemura T, Maki S, Wieslander J, Takekoshi Y, et al. Type IV Collagen a5 chain: normal distribution and abnormalities in X-linked Alport syndrome revealed by monoclonal antibody. Am J Pathol. 1994;144:986–96.
23. Peissel B, Geng L, Kalluri R, Kashtan C, Rennke HG, Gallo GR, et al. Comparative distribution of the a1(IV), a5(IV) and a6(IV) collagen chains in normal human adult and fetal tissues and in kidneys from X-linked Alport syndrome patients. J Clin Invest. 1995;96:1948–57.
24. Cheong HI, Kashtan CE, Kim Y, Kleppel MM, Michael AF. Immunohistologic studies of type IV collagen in anterior lens capsules of patients with Alport syndrome. Lab Invest. 1994;70:553–7.
25. Cosgrove D, Kornak JM, Samuelson G. Expression of basement membrane type IV collagen chains during postnatal development in the murine cochlea. Hear Res. 1996;100:21–32.
26. Zehnder AF, Adams JC, Santi PA, Kristiansen AG, Wacharasindhu C, Mann S, et al. Distribution of type IV collagen in the cochlea in Alport syndrome. Arch Otolaryngol Head Neck Surg. 2005;131:1007–13.
27. Gunwar S, Ballester F, Noelken ME, Sado Y, Ninomiya Y, Hudson BG. Glomerular basement membrane. Identification of a novel disulfide-cross-linked network of alpha3, alpha4, and alpha5 chains of type IV collagen and its implications for the pathogenesis of Alport syndrome. J Biol Chem. 1998;273(15):8767–75.
28. Zeisberg M, Khurana M, Rao VH, Cosgrove D, Rougier JP, Werner MC, et al. Stage-specific action of matrix metalloproteinases influences progressive hereditary kidney disease. PLoS Med. 2006;3(4):e100.
29. Gyoneva L, Segal Y, Dorfman KD, Barocas VH. Mechanical response of wild-type and Alport murine lens capsules during osmotic swelling. Exp Eye Res. 2013;113:87–91.
30. Meehan DT, Delimont D, Cheung L, Zallocchi M, Sansom SC, Holzclaw JD, et al. Biomechanical strain causes maladaptive gene regulation, contributing to Alport glomerular disease. Kidney Int. 2009;76(9):968–76.
31. Storey H, Savige J, Sivakumar V, Abbs S, Flinter FA. COL4A3/COL4A4 mutations and features in individuals with autosomal recessive Alport syndrome. J Am Soc Nephrol. 2013;24(12):1945–54.
32. Chiara F, Laura D, Rossella T, Dorella DP, Sandro F, Giorgia G, et al. Unbiased next generation sequencing analysis confirms the existence of autosomal dominant Alport syndrome in a relevant fraction of cases. Clin Genet. 2014;86(3):252–7.
33. Pescucci C, Mari F, Longo I, Vogiatzi P, Caselli R, Scala E, et al. Autosomal-dominant Alport syndrome: natural history of a disease due to COL4A3 or COL4A4 gene. Kidney Int. 2004;65(5):1598–603.
34. Lemmink HH, Nillesen WN, Mochizuki T, Schroder CH, Brunner HG, van Oost BA, et al. Benign familial hematuria due to mutation of the type IV collagen alpha4 gene. J Clin Invest. 1996;98(5):1114–8.
35. Crockett DK, Pont-Kingdon G, Gedge F, Sumner K, Seamons R, Lyon E. The Alport syndrome COL4A5 variant database. Hum Mutat. 2010;31(8):E1652–7.
36. Jais JP, Knebelmann B, Giatras I, De Marchi M, Rizzoni G, Renieri A, et al. X-linked Alport syndrome: natural history in 195 families and genotype-phenotype

correlations in males. J Am Soc Nephrol. 2000;11: 649–57.
37. Gross O, Netzer KO, Lambrecht R, Seibold S, Weber M. Meta-analysis of genotype-phenotype correlation in X-linked Alport syndrome: impact on clinical counseling. Nephrol Dial Transplant. 2002;17:1218–27.
38. Jais JP, Knebelmann B, Giatras I, De Marchi M, Rizzoni G, Renieri A, et al. X-linked Alport syndrome: natural history and genotype-phenotype correlations in girls and women belonging to 195 families: a "European Community Alport Syndrome Concerted Action" study. J Am Soc Nephrol. 2003; 14:2603–10.
39. Marcocci E, Uliana V, Bruttini M, Artuso R, Silengo MC, Zerial M, et al. Autosomal dominant Alport syndrome: molecular analysis of the COL4A4 gene and clinical outcome. Nephrol Dial Transplant. 2009;24(5):1464–71.
40. Gubler M, Levy M, Broyer M, Naizot C, Gonzales G, Perrin D, et al. Alport's syndrome: a report of 58 cases and a review of the literature. Am J Med. 1981;70:493–505.
41. Kashtan CE, Ding J, Gregory M, Gross O, Heidet L, Knebelmann B, et al. Clinical practice recommendations for the treatment of Alport syndrome: a statement of the Alport Syndrome Research Collaborative. Pediatr Nephrol. 2013;28(1):5–11.
42. Rheault MN. Women and Alport syndrome. Pediatr Nephrol. 2012;27(1):41–6.
43. Grunfeld J-P, Noel LH, Hafez S, Droz D. Renal prognosis in women with hereditary nephritis. Clin Nephrol. 1985;23:267–71.
44. Guo C, Van Damme B, Vanrenterghem Y, Devriendt K, Cassiman JJ, Marynen P. Severe alport phenotype in a woman with two missense mutations in the same COL4A5 gene and preponderant inactivation of the X chromosome carrying the normal allele. J Clin Invest. 1995;95(4):1832–7.
45. Iijima K, Nozu K, Kamei K, Nakayama M, Ito S, Matsuoka K, et al. Severe Alport syndrome in a young woman caused by a t(X;1)(q22.3;p36.32) balanced translocation. Pediatr Nephrol. 2010;25(10):2165–70.
46. Rheault MN, Kren SM, Hartich LA, Wall M, Thomas W, Mesa HA, et al. X-inactivation modifies disease severity in female carriers of murine X-linked Alport syndrome. Nephrol Dial Transplant. 2010;25(3):764–9.
47. Wester DC, Atkin CL, Gregory MC. Alport syndrome: clinical update. J Am Acad Audiol. 1995;6:73–9.
48. Kleppel MM, Santi PA, Cameron JD, Wieslander J, Michael AF. Human tissue distribution of novel basement membrane collagen. Am J Pathol. 1989;134:813–25.
49. Cosgrove D, Samuelson G, Meehan DT, Miller C, McGee J, Walsh EJ, et al. Ultrastructural, physiological, and molecular defects in the inner ear of a gene-knockout mouse model of autosomal Alport syndrome. Hear Res. 1998;121:84–98.
50. Harvey SJ, Mount R, Sado Y, Naito I, Ninomiya Y, Harrison R, et al. The inner ear of dogs with X-linked nephritis provides clues to the pathogenesis of hearing loss in X-linked Alport syndrome. Am J Pathol. 2001;159(3):1097–104.
51. Merchant SN, Burgess BJ, Adams JC, Kashtan CE, Gregory MC, Santi PA, et al. Temporal bone histopathology in alport syndrome. Laryngoscope. 2004; 114(9):1609–18.
52. Gratton MA, Rao VH, Meehan DT, Askew C, Cosgrove D. Matrix metalloproteinase dysregulation in the stria vascularis of mice with Alport syndrome: implications for capillary basement membrane pathology. Am J Pathol. 2005;166(5):1465–74.
53. Nielsen CE. Lenticonus anterior and Alport's syndrome. Arch Ophthalmol. 1978;56:518–30.
54. Colville DJ, Savige J. Alport syndrome. A review of the ocular manifestations. Ophthalmic Genet. 1997;18(4):161–73.
55. Streeten BW, Robinson MR, Wallace R, Jones DB. Lens capsule abnormalities in Alport's syndrome. Arch Ophthalmol. 1987;105:1693–7.
56. Kato T, Watanabe Y, Nakayasu K, Kanai A, Yajima Y. The ultrastructure of the lens capsule abnormalities in Alport's syndrome. Jpn J Ophthalmol. 1998;42:401–5.
57. Perrin D, Jungers P, Grunfeld JP, Delons S, Noel LH, Zenatti C. Perimacular changes in Alport's syndrome. Clin Nephrol. 1980;13:163–7.
58. Rhys C, Snyers B, Pirson Y. Recurrent corneal erosion associated with Alport's syndrome. Kidney Int. 1997;52:208–11.
59. Burke JP, Clearkin LG, Talbot JF. Recurrent corneal epithelial erosions in Alport's syndrome. Acta Ophthalmol. 1991;69:555–7.
60. Teekhasaenee C, Nimmanit S, Wutthiphan S, Vareesangthip K, Laohapand T, Malasitr P, et al. Posterior polymorphous dystrophy and Alport syndrome. Ophthalmology. 1991;98:1207–15.
61. Antignac C, Heidet L. Mutations in Alport syndrome associated with diffuse esophageal leiomyomatosis. Contrib Nephrol. 1996;117:172–82.
62. Antignac C, Knebelmann B, Druout L, Gros F, Deschenes G, Hors-Cayla M-C, et al. Deletions in the COL4A5 collagen gene in X-linked Alport syndrome: characterization of the pathological transcripts in non-renal cells and correlation with disease expression. J Clin Invest. 1994;93:1195–207.
63. Zhou J, Mochizuki T, Smeets H, Antignac C, Laurila P, de Paepe A, et al. Deletion of the paired a5(IV) and a6(IV) collagen genes in inherited smooth muscle tumors. Science. 1993;261:1167–9.
64. Segal Y, Peissel B, Renieri A, de Marchi M, Ballabio A, Pei Y, et al. LINE-1 elements at the sites of molecular rearrangements in Alport syndrome-diffuse leiomyomatosis. Am J Hum Genet. 1999;64:62–9.
65. Jonsson JJ, Renieri A, Gallagher PG, Kashtan CE, Cherniske EM, Bruttini M, et al. Alport syndrome, mental retardation, midface hypoplasia, and elliptocytosis: a new X linked contiguous gene deletion syndrome? J Med Genet. 1998;35(4):273–8.

66. Kashtan CE, Segal Y, Flinter F, Makanjuola D, Gan JS, Watnick T. Aortic abnormalities in males with Alport syndrome. Nephrol Dial Transplant. 2010; 25(11):3554–60.
67. Kashtan CE, Kleppel MM, Gubler MC. Immunohistologic findings in Alport syndrome. Contrib Nephrol. 1996;117:142–53.
68. Martin P, Heiskari N, Zhou J, Leinonen A, Tumelius T, Hertz JM, et al. High mutation detection rate in the COL4A5 collagen gene in suspected Alport syndrome using PCR and direct DNA sequencing. J Am Soc Nephrol. 1998;9:2291–301.
69. Gross O, Schulze-Lohoff E, Koepke ML, Beirowski B, Addicks K, Bloch W, et al. Antifibrotic, nephroprotective potential of ACE inhibitor vs AT1 antagonist in a murine model of renal fibrosis. Nephrol Dial Transplant. 2004;19(7):1716–23.
70. Gross O, Beirowski B, Koepke ML, Kuck J, Reiner M, Addicks K, et al. Preemptive ramipril therapy delays renal failure and reduces renal fibrosis in COL4A3-knockout mice with Alport syndrome. Kidney Int. 2003;63(2):438–46.
71. Gross O, Koepke ML, Beirowski B, Schulze-Lohoff E, Segerer S, Weber M. Nephroprotection by antifibrotic and anti-inflammatory effects of the vasopeptidase inhibitor AVE7688. Kidney Int. 2005;68: 456–63.
72. Sayers R, Kalluri R, Rodgers KD, Shield CF, Meehan DT, Cosgrove D. Role for transforming growth factor-beta 1 in Alport renal disease progression. Kidney Int. 1999;56:1662–73.
73. Ninichuk V, Gross O, Reichel C, Kandoga A, Pawar RD, Ciubar R, et al. Delayed chemokine receptor 1 blockade prolongs survival in collagen 4A3-deficient miche with Alport disease. J Am Soc Nephrol. 2005;16:977–85.
74. Zeisberg M, Bottiglio C, Kumar N, Maeshima Y, Strutz F, Muller GA, et al. Bone morphogenic protein-7 inhibits progression of chronic renal fibrosis associated with two genetic mouse models. Am J Physiol Renal Physiol. 2003;285(6):F1060–7.
75. Sugimoto H, Mundel TM, Sund M, Xie L, Cosgrove D, Kalluri R. Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. Proc Natl Acad Sci U S A. 2006;103(19):7321–6.
76. Chen D, Jefferson B, Harvey SJ, Zheng K, Gartley CJ, Jacobs RM, et al. Cyclosporine a slows the progressive renal disease of alport syndrome (X-linked hereditary nephritis): results from a canine model. J Am Soc Nephrol. 2003;14(3):690–8.
77. Charbit M, Gubler MC, Dechaux M, Gagnadoux MF, Grunfeld JP, Niaudet P. Cyclosporin therapy in patients with Alport syndrome. Pediatr Nephrol (Berlin, Germany). 2007;22(1):57–63.
78. Massella L, Muda AO, Legato A, Di Zazzo G, Giannakakis K, Emma F. Cyclosporine A treatment in patients with Alport syndrome: a single-center experience. Pediatr Nephrol (Berlin, Germany). 2010;25(7):1269–75.
79. Grodecki KM, Gains MJ, Baumal R, Osmond DH, Cotter B, Valli VE, Jacobs RM. Treatment of X-linked hereditary nephritis in Samoyed dogs with angiotensin converting enzyme inhibitor. J Comp Pathol. 1997;117:209–25.
80. Cohen EP, Lemann J. In hereditary nephritis angiotensin-converting enzyme inhibition decreases proteinuria and may slow the rate of progression. Am J Kidney Dis. 1996;27:199–203.
81. Proesmans W, Van Dyck M. Enalapril in children with Alport syndrome. Pediatr Nephrol. 2004;19(3): 271–5.
82. Webb NJ, Lam C, Shahinfar S, Strehlau J, Wells TG, Gleim GW, et al. Efficacy and safety of losartan in children with Alport syndrome – results from a subgroup analysis of a prospective, randomized, placebo- or amlodipine-controlled trial. Nephrol Dial Transplant. 2011;26(8):2521–6.
83. Webb NJ, Shahinfar S, Wells TG, Massaad R, Gleim GW, McCrary Sisk C, et al. Losartan and enalapril are comparable in reducing proteinuria in children with Alport syndrome. Pediatr Nephrol. 2013;28(5): 737–43.
84. Gross O, Licht C, Anders HJ, Hoppe B, Beck B, Tonshoff B, et al. Early angiotensin-converting enzyme inhibition in Alport syndrome delays renal failure and improves life expectancy. Kidney Int. 2012;81(5):494–501.
85. Gross O, Friede T, Hilgers R, Gorlitz A, Gavenis K, Ahmed R, et al. Safety and efficacy of the ACE-inhibitor Ramipril in Alport syndrome: the double-blind, randomized, placebo-controlled, multicenter phase III EARLY PRO-TECT Alport trial in pediatric patients. ISRN Pediatr. 2012;2012:436046.
86. Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F. Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. J Am Soc Nephrol. 2013;24(3):364–75.
87. Wuhl E, Trivelli A, Picca S, Litwin M, Peco-Antic A, Zurowska A, et al. Strict blood-pressure control and progression of renal failure in children. N Engl J Med. 2009;361(17):1639–50.
88. Temme J, Kramer A, Jager KJ, Lange K, Peters F, Muller GA, et al. Outcomes of male patients with Alport syndrome undergoing renal replacement therapy. Clin J Am Soc Nephrol. 2012;7(12):1969–76.
89. Gross O, Weber M, Fries JW, Muller GA. Living donor kidney transplantation from relatives with mild urinary abnormalities in Alport syndrome: long-term risk, benefit and outcome. Nephrol Dial Transplant. 2009;24(5):1626–30.
90. Kashtan CE. Renal transplantation in patients with Alport syndrome. Pediatr Transplant. 2006;10: 651–7.
91. Brainwood D, Kashtan C, Gubler MC, Turner AN. Targets of alloantibodies in Alport antiglomerular basement membrane disease after renal transplantation. Kidney Int. 1998;53:762–6.
92. Dehan P, Van Den Heuvel LPWJ, Smeets HJM, Tryggvason K, Foidart J-M. Identification of

post-transplant anti-a5(IV) collagen alloantibodies in X-linked Alport syndrome. Nephrol Dial Transplant. 1996;11:1983–8.
93. Kalluri R, van den Heuvel LP, Smeets HJM, Schroder CH, Lemmink HH, Boutaud A, et al. A COL4A3 gene mutation and post-transplant anti-a3(IV) collagen alloantibodies in Alport syndrome. Kidney Int. 1995;47:1199–204.
94. Wang XP, Fogo AB, Colon S, Giannico G, Abul-Ezz SR, Miner JH, et al. Distinct epitopes for anti-glomerular basement membrane Alport alloantibodies and goodpasture autoantibodies within the noncollagenous domain of {alpha}3(IV) collagen: a janus-faced antigen. J Am Soc Nephrol. 2005;16: 3563–71.
95. Marks MI, Drummond KN. Benign familial hematuria. Pediatrics. 1969;44:590–3.
96. McConville JM, West CD, McAdams AJ. Familial and nonfamilial benign hematuria. J Pediatr. 1966; 69:207–14.
97. Pardo V, Berian MG, Levi DF, Strauss J. Benign primary hematuria: clinicopathologic study of 65 patients. Am J Med. 1979;67:817–22.
98. Savige J, Rana K, Tonna S, Buzza M, Dagher H, Wang YY. Thin basement membrane nephropathy. Kidney Int. 2003;64(4):1169–78.
99. Haas M. Thin glomerular basement membrane nephropathy: incidence in 3471 consecutive renal biopsies examined by electron microscopy. Arch Pathol Lab Med. 2006;130(5):699–706.
100. Dische FE, Anderson VER, Keane SJ, Taube D, Bewick M, Parsons V. Incidence of thin membrane nephropathy: morphometric investigation of a population sample. J Clin Pathol. 1990;43:457–60.
101. Tryggvason K, Patrakka J. Thin basement membrane nephropathy. J Am Soc Nephrol. 2006;17(3):813–22.
102. Gregory MC. The clinical features of thin basement membrane nephropathy. Semin Nephrol. 2005;25(3): 140–5.
103. Auwardt R, Savige J, Wilson D. A comparison of the clinical and laboratory features of thin basement membrane disease (TBMD) and IgA glomerulonephritis (IgA GN). Clin Nephrol. 1999;52(1):1–4.
104. Nieuwhof CM, de Heer F, de Leeuw P, van Breda Vriesman PJ. Thin GBM nephropathy: premature glomerular obsolescence is associated with hypertension and late onset renal failure. Kidney Int. 1997;51(5):1596–601.
105. van Paassen P, van Breda Vriesman PJ, van Rie H, Tervaert JW. Signs and symptoms of thin basement membrane nephropathy: a prospective regional study on primary glomerular disease-The Limburg Renal Registry. Kidney Int. 2004;66(3):909–13.
106. Pettersson E, Tornroth T, Wieslander J. Abnormally thin glomerular basement membrane and the Goodpasture epitope. Clin Nephrol. 1990;33:105–9.
107. Vogler C, McAdams AJ, Homan SM. Glomerular basement membrane and lamina densa in infants and children: an ultrastructural evaluation. Pediatr Pathol. 1987;7:527–34.
108. Steffes MW, Barbosa J, Basgen JM, Sutherland DER, Najarian JS, Mauer SM. Quantitative glomerular morphology of the normal human kidney. Kidney Int. 1983;49:82–6.
109. Dische FE. Measurement of glomerular basement membrane thickness and its application to the diagnosis of thin-membrane nephropathy. Arch Pathol Lab Med. 1992;116:43–9.
110. Tiebosch ATMG, Frederik PM, van Breda Vriesman PJC, Mooy JMV, van Rie H, van de Wiel TWM, et al. Thin-basement-membrane nephropathy in adults with persistent hematuria. N Engl J Med. 1989;320:14–8.
111. Milanesi C, Rizzoni G, Braggion F, Galdiolo D. Electron microscopy for measurement of glomerular basement membrane width in children with benign familial hematuria. Appl Pathol. 1984;2:199–204.
112. Blumenthal SS, Fritsche C, Lemann J. Establishing the diagnosis of benign familial hematuria: the importance of examining the urine sediment of family members. JAMA. 1988;259:2263–6.
113. Plaisier E, Gribouval O, Alamowitch S, Mougenot B, Prost C, Verpont MC, et al. COL4A1 mutations and hereditary angiopathy, nephropathy, aneurysms, and muscle cramps. N Engl J Med. 2007;357(26): 2687–95.
114. Plaisier E, Chen Z, Gekeler F, Benhassine S, Dahan K, Marro B, et al. Novel COL4A1 mutations associated with HANAC syndrome: a role for the triple helical CB3[IV] domain. Am J Med Genet A. 2010;152A(10):2550–5.
115. Alamowitch S, Plaisier E, Favrole P, Prost C, Chen Z, Van Agtmael T, et al. Cerebrovascular disease related to COL4A1 mutations in HANAC syndrome. Neurology. 2009;73(22):1873–82.
116. Poschl E, Schlotzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. Development. 2004;131(7):1619–28.