Alport Syndrome and Other Type IV Collagen Disorders

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Introduction

Several forms of familial glomerular hematuria syndromes result from genetic variants that affect type IV collagen, the major collagenous constituent of glomerular basement membranes (GBM): Alport syndrome (AS) and hereditary angiopathy with nephropathy, aneurysms and cramps (HANAC) syndrome. Persistent hematuria is a cardinal feature of each of these disorders. Variants in any of three type IV collagen genes, *COL4A3, COL4A4* or *COL4A5* can cause AS, which is characterized 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. Heterozygous variants in these genes are also significant and link to a wider spectrum of kidney disease [1–3]. Variants in *COL4A3, COL4A4 or COL4A5* [4] account for about 30–50% of children with isolated glomerular hematuria seen in pediatric nephrology clinics [5–8]. HANAC syndrome arises from variants in *COL4A1* [9].

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Alport Syndrome

Introduction

The first description of a family with inherited hematuria appeared in 1902 in a report by Guthrie [10]. Subsequent monographs about this family by Hurst in 1923 [11] and Alport in 1927 [12] established that affected individuals in this family, particularly males, developed deafness and uremia. The advent of electron microscopy led to the discovery of unique GBM abnormalities in patients with AS [13–15], setting the stage for the histochemical [16–18] and genetic [19, 20] studies that resulted in the identification of disease causing variants in *COL4A5* [20] followed by *COL4A3* and *COL4A4* [21, 22]. AS occurs in approximately 1:50,000 live births and accounts for 1.3% and 0.4% of pediatric and adult end-stage kidney disease (ESKD) patients in the United States, respectively [23].

F. Schaefer, L. A. Greenbaum (eds.), *Pediatric Kidney Disease*,
https://doi.org/10.1007/978-3-031-11665-0_16

Etiology and Pathogenesis

Type IV Collagen Proteins, Tissue Distribution and Genes

Six chains 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′ head-to-head fashion, separated by sequences of varying length containing regulatory elements [24, 25]. All type IV collagen chains share several basic structural features: a major collagenous domain of approximately 1400 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) [26]. 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 [27].

$\alpha1_2\alpha2$(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 [28, 29]. $\alpha5_2\alpha6$(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 [30–32].

Pathogenic variants 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 loss of function variants (deletions, frame shift variants, premature stop codons), then the other collagen chains are degraded and no α3α4α5(IV) trimers are deposited in basement membranes [33]. In this case, the embryonic $\alpha1_2\alpha2$(IV) network persists. Missense variants, 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 considered more resistant to proteases and therefore mechanical strain than the $\alpha1_2\alpha2$(IV) network [33, 34]. In support of this network being mechanically stronger, absence of the α3α4α5(IV) network leads to increased distensibility in the lens capsule when tested in experimental models of AS [35]. Indeed, the glomerular capillary walls of AS patients may also be mechanically weak and provoke pathologic stretch-related responses in glomerular cells [36].

Genetics

AS is described in three genetic forms: X-linked (XLAS), autosomal recessive (ARAS) and autosomal dominant (ADAS), although opinions vary as to how a single gene can cause both recessive and dominant disease (Table 16.1). XLAS, caused by variants in *COL4A5,* was classically thought to account for approximately 80% of AS patients while ARAS, caused by variants in both alleles of *COL4A3* or *COL4A4,* accounted for about 15% of the AS population. Affected males with XLAS are hemizygous and carry a single abnormal *COL4A5* allele, while affected females are heterozygous with normal and abnormal alleles. Individuals with ARAS may be either homozygous, with identical variants in both

Table 16.1 Familial glomerular hematuria due to type IV collagen variants

	Genetic locus	Protein product	Kidney manifestations	Kidney failure	GBM ultrastructure	Extrakidney 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* *COL4A4* (biallelic or digenic)	α3(IV) α4(IV)	Hematuria Proteinuria Hypertension	All males and females	Thinning (early) Lamellation (late)	Deafness Lenticonus Perimacular flecks
Autosomal dominant	*COL4A3* *COL4A4* (heterozygous)	α3(IV) α4(IV)	Hematuria Proteinuria Hypertension	Males and females (late)	Thinning (early) Lamellation (late)	Deafness
HANAC syndrome						
Autosomal dominant	*COL4A1*	α1(IV)	Hematuria Cysts CKD	?	Normal	Arterial aneurysms Muscle cramps

Abbreviations: *GBM* glomerular basement membrane; *CKD* chronic kidney disease

alleles of the affected gene or they may be compound heterozygotes, with different variants in the two alleles or even demonstrate digenic inheritance with one variant in *COL4A3* and the other in *COL4A4* [37, 38]. With the advent of next generation sequencing, studies are suggesting a higher percentage of patients with ADAS, up to 31% in one report [39]. ADAS is used by some clinicians to describe heterozygous variants in *COL4A3* or *COL4A4* with a progressive clinical course [40]. It is not clear why some individuals develop a progressive nephropathy while others have a slower or unremarkable clinical course; this may relate to the presence of co-segregating genetic modifiers [41].

Over 2500 pathogenic variants have been identified in the *COL4A5* gene in patients with XLAS [42]. Variants can be found along the entire 51 exons of the gene without identified hot spots. About 10–15% of *COL4A5* variants occur as spontaneous events; therefore, a family history of kidney disease is not required for a diagnosis of XLAS. A range of variants have been described: large rearrangements (~20%), small deletions and insertions (~20%), missense variants altering a glycine residue in the collagenous domain of α5(IV) (30%), other missense variants (~8%), nonsense variants (~5%) and splice-site variants (~15%) [43]. The type of *COL4A5* variant has a significant impact on the course of XLAS in affected males [43–45]. In males with a large deletion, nonsense variant or an indel causing a reading frame shift, the risk of developing kidney failure before age 30 is 90%. In contrast, progression to kidney failure before age 30 occurs in 70% and 50% of patients with splice-site and missense variants, respectively [43]. In addition, XLAS patients with 5′ glycine missense variants demonstrate a more severe phenotype than those with 3′ glycine variants [44]. In contrast to males with XLAS, a statistical relationship between *COL4A5* genotype and kidney phenotype has not been demonstrated in females with XLAS [46].

Clinical Manifestations

Males with XLAS and ARAS inevitably develop kidney failure at a rate that is influenced by genotype [37, 43, 45]. While most females with XLAS have non-progressive or slowly progressive kidney disease, a significant minority demonstrates progression to kidney failure [47]. The course of ARAS is similar in females and males [37]. In general, patients

with ADAS progress less rapidly than patients with XLAS or ARAS and are less likely to have extra-kidney manifestations [48].

Kidney Phenotype

Persistent microscopic hematuria (MH) occurs in all males with AS, regardless of genetic type, and is probably present from early infancy. Approximately 95% of heterozygous females with XLAS have persistent or intermittent MH [46], and 100% of females with ARAS have persistent MH. Gross hematuria is not unusual in boys and girls with Alport syndrome, occurring at least once in approximately 60% of affected males [43, 49].

In males with XLAS, and in males and females with ARAS, proteinuria typically becomes detectable in late childhood or early adolescence and progresses from microalbuminuria to overt proteinuria [50]. In one large cohort of females with XLAS, 75% had proteinuria, although the timing of onset was not investigated [46].

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 kidney replacement therapy, with 50% of untreated males reaching kidney failure by age 25, 80% by age 40 and 100% by age 60 [43]. The timing of kidney failure in patients with ARAS is similar to XLAS males, although ARAS patients with normal kidney function in their 30's and 40's have been reported [37]. In patients with ADAS, the age at which 50% of patients have progressed to kidney failure is approximately 50 years, or twice as long as XLAS males [48].

Females who are heterozygous for *COL4A5* variants demonstrate widely variable disease outcomes, with some women demonstrating only lifelong asymptomatic hematuria while others develop chronic progressive kidney disease including kidney failure [51]. About 12% of XLAS females reach kidney failure by age 45, 30% by age 60 and 40% by age 80 [46]. The explanation for the wide variability in outcomes for XLAS females is uncertain, but likely multifactorial. Risk factors for kidney failure in XLAS females include proteinuria and sensorineural deafness [46, 52]. 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 kidney disease progression in XLAS females [53, 54]. 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 [55].

AS 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 kidney function. In Phase II, microalbuminuria followed by overt proteinuria is superimposed on hematuria, but the glomerular filtration rate (GFR) remains normal. Patients in Phase III exhibit declining GFR in addition to hematuria and proteinuria, and those in Phase IV have kidney failure. 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 genetic variant, at least in males with XLAS. Patients with *COL4A5* variants that prevent production of any functional protein (deletions, nonsense variants) proceed through these phases more rapidly than those whose variants allow synthesis of a functional, albeit abnormal, protein (some missense variants). Females with XLAS can be viewed as passing through the same phases as males, although the rate of progression is typically slower, and the journey to kidney failure 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. Affected individuals benefit from hearing aids since the deficit usually does not exceed 60–70 dB and speech discrimination is preserved. Sensorineural hearing loss (SNHL) is present in 50% of males with XLAS by approximately age 15, 75% by age 25, and 90% by age 40 [43]. Like the effect on kidney disease progression, missense variants in *COL4A5* are associated with an attenuated risk of hearing loss. The risk of SNHL before age 30 is 60% in patients with missense variants, while the risk of SNHL before age 30 is 90% in those with other types of variants [43]. 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 [46]. SNHL is also common in ARAS, with approximately 66% of individuals affected [37].

The SNHL in AS has been localized to the cochlea [56]. 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 [57–59]. However, these chains have not been detected in the cochleae of ARAS mice [58], XLAS dogs [59] or men with XLAS [32]. 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 [60]. 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 [61].

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 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 variants that interfere with the formation or deposition of α3α4α5(IV) trimers prevent expression of these chains in the eye [30, 57]. Anterior lenticonus, which is considered virtually pathognomonic for AS [62], is absent at birth and manifests during the second and third decades of life in ~13–25% of affected individuals [43, 63]. 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 [64, 65]. Anterior lenticonus has been described only rarely in heterozygous females with *COL4A5* variants [47]. Dot-fleck retinopathy, a characteristic alteration of retinal pigmentation concentrated in the perimacular region [66], is also common in AS patients and does not appear to be associated with any abnormality in vision [43]. Recurrent corneal erosions [67, 68] and posterior polymorphous dystrophy, manifested by clear vesicles on the posterior surface of the cornea [69], 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 [70]. Affected individuals carry X-chromosomal deletions that involve the *COL4A5* gene and terminate within the second intron of the adjacent *COL4A6* gene [71–73]. The genotype-phenotype relationship in this disorder is uncertain because deletions in this region may occur without associated leiomyomas, and conversely some families with XLAS and leiomyomas do not have deletions involving *COL4A6* [74]. 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 may develop 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 [75]. 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 [76].

Kidney Pathology

Children with AS typically show limited kidney parenchymal changes by light microscopy before 5 years of age. Older patients may have mesangial hypercellularity and matrix expansion. 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 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. The classic ultrastructural appearance is diffuse thickening of the glomerular capillary wall, accompanied by "basket-weave" transformation; intramembranous cellular components, which have been described as podocyte protrusions; scalloping of the epithelial surface of the GBM; and effacement of podocyte foot-processes (Fig. 16.1) [77]. 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 predominantly normal-appearing GBM, focal GBM attenuation, diffuse GBM attenuation, focal thickening/basket-weaving, or diffuse basket-

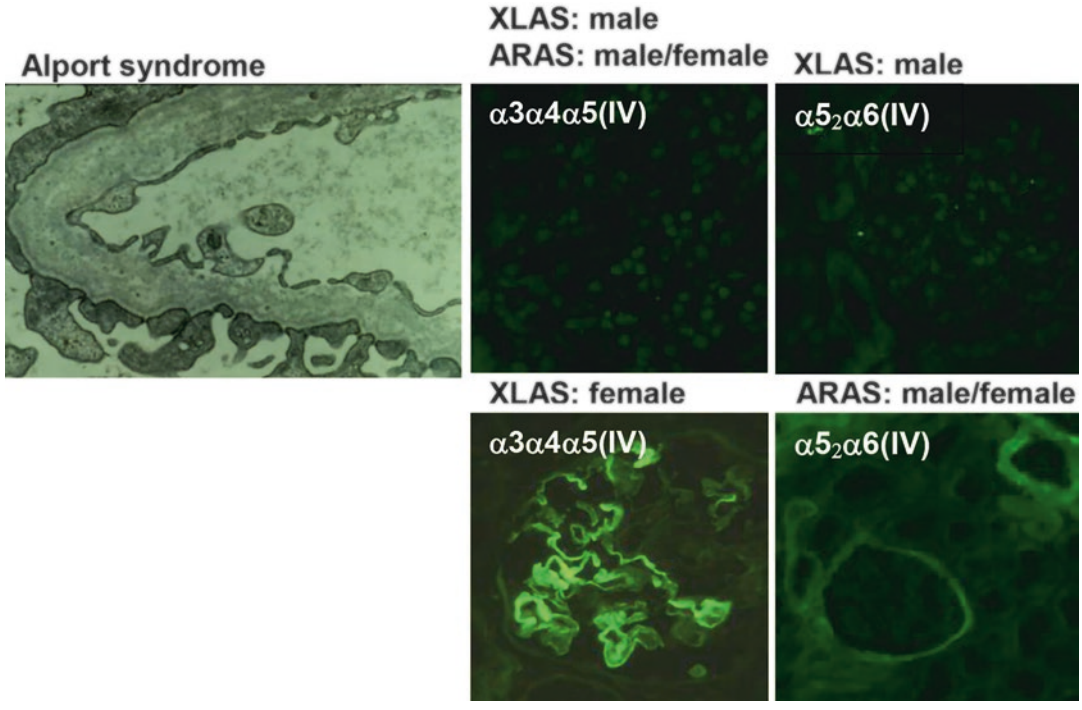

Fig. 16.1 Typical findings on electron microscopy and type IV collagen immunostaining for α5(IV) in Alport syndrome. Abbreviations: *XLAS* X-linked Alport syndrome; *ARAS* autosomal recessive Alport syndrome

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 GBM changes 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* variants, have been described. Although these represent a minority of Alport patients and families, they are also seen in individuals with heterozygous variants and in such patients there is an association with focal segmental glomerulosclerosis (FSGS) [1, 2]. Indeed, patients with a diagnosis of FSGS should have careful evaluation of GBM ultrastructure and, if defects are identified, genetic testing for Alport gene variants is warranted since a diagnosis of AS will enable further phenotypic evaluation in the individual as well as testing in other family members.

Routine immunofluorescence microscopy in patients with AS is normal or shows nonspecific deposition of immunoproteins. In contrast, specific immunostaining for type IV collagen α chains is frequently diagnostic, and can distinguish between XLAS and ARAS (Fig. 16.1). The utility of this approach derives from the fact that most disease-causing variants in AS alter the expression of the α3α4α5(IV) and $\alpha5_2\alpha6$(IV) trimers in kidney basement membranes. Most *COL4A5* variants prevent expression of both trimer forms in the kidney, so that in about 80% of XLAS males immunostaining of kidney biopsy specimens for α3(IV), α4(IV) and α5(IV) chains is completely negative [78]. About 60–70% of XLAS females exhibit mosaic expression of these chains, while in the remainder immunostaining for these chains is normal. The biallelic variants in *COL4A3* and *COL4A4* that cause ARAS often prevent expression of α3α4α5(IV) trimers, but have no effect on expression of $\alpha5_2\alpha6$(IV) trimers. In kidney 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 α3α4α5(IV) trimers, Bowman's capsules, distal tubular basement membranes and 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 normal kidney basement membrane immunostaining for α3(IV), α4(IV) and α5(IV) chains.

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

Diagnosis and Differential Diagnosis

AS is 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 (Fig. 16.2).

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 kidney failure strongly suggests a diagnosis of heterozygous *COL4A3/4* variants (Fig. 16.2). 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 and familial IgA nephropathy. However, there may also be overlap with heterozygous Alport syndrome and a range of glomerular pathologies; large genetic sequencing studies will help to identify these disease group intersections.

When family history for hematuria is negative, the differential diagnosis of isolated glomerular hematuria, or hematuria associated with proteinuria includes AS, IgA nephropathy, C3

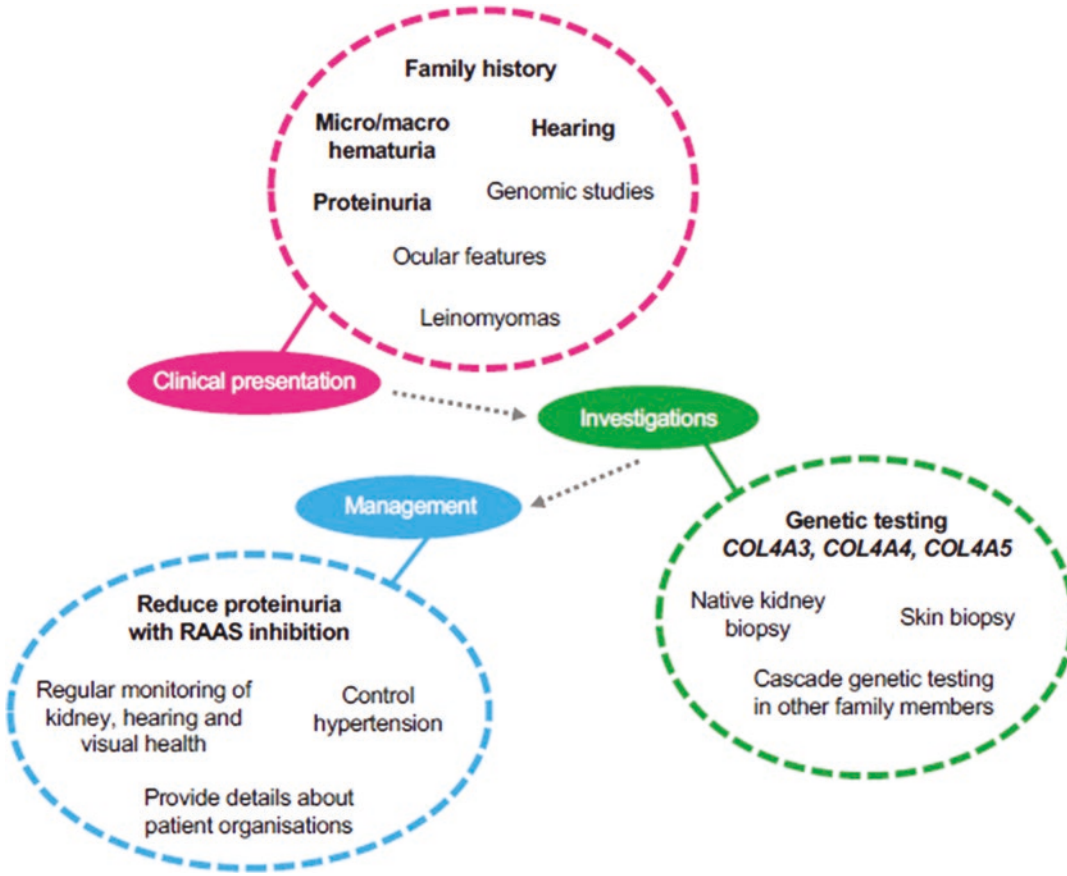

Fig. 16.2 Clinical presentation, diagnosis and management of Alport syndrome. Patients can present with a variety of clinical presentations, which should prompt investigation to confirm a diagnosis of Alport syndrome. Genetic testing for the Alport genes *COL4A3, COL4A4* and *COL4A5* is widely available and is the gold standard for diagnosis. Management includes the use of renin-angiotensin-aldosterone (RAAS) pathway inhibitors

glomerulopathy, membranous nephropathy, lupus nephritis, postinfectious glomerulonephritis, Henoch-Schönlein nephritis, and many other entities. Some of these conditions will be strongly suspected based on 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.

Genetic testing is the gold standard for diagnosing AS. Additional tissue studies are appropriate when clinical and pedigree information and genetic testing does not allow a diagnosis AS. Therefore, several options are available for confirming a diagnosis of AS, including genetic analysis, skin biopsy and kidney biopsy. Genetic analysis using Sanger sequencing is capable of identifying *COL4A5* variants in 80–90% of males with XLAS [79]. High variant detection rates in *COL4A3* and *COL4A4* in patients with ARAS are

also possible, particularly if there is parental consanguinity. Commercial genetic testing for variants in *COL4A3, COL4A4*, and *COL4A5* is available. Next generation sequencing, which allows simultaneous analysis of *COL4A3, COL4A4* and *COL4A5*, now replaces Sanger sequencing as the preferred approach. If further investigation is required, skin biopsy is often utilized as the initial invasive diagnostic procedure in patients suspected of AS it is less invasive and expensive than a kidney biopsy. On skin biopsy, the majority of subjects with XLAS will display abnormal expression of the α5(IV) chain in 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. Kidney biopsy would then provide the opportunity to diagnose other diseases, to examine type IV collagen α chain expression in kidney basement membranes, and to evaluate GBM at the ultrastructural level.

Treatment

The goal of treatment in AS is to slow the progression of kidney disease and delay the need for dialysis or transplantation. Several therapeutic approaches have demonstrated efficacy in murine ARAS, including angiotensin blockade [80–82], inhibition of TGFβ-1 [83], chemokine receptor 1 blockade [84], administration of bone morphogenic protein-7 [85], suppression of matrix metalloproteinases [34] and bone marrow transplantation [86]. Cyclosporine therapy slowed progression of kidney disease in a canine model of AS, but human studies have demonstrated significant nephrotoxicity and adverse effects and this treatment is not recommended [87–89]. Angiotensin converting enzyme (ACE) inhibition also prolonged survival in a canine XLAS model [90]. Uncontrolled studies in human AS subjects have shown that ACE inhibition can reduce proteinuria, at least transiently [91, 92]. 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 [93]. An extension of this study showed comparable efficacy of either enalapril or losartan in reducing proteinuria in children with AS [94]. A report from the European Alport Registry, which includes 283 patients over 20 years, compared kidney outcomes in AS patients treated with ACE inhibition at various time points: at onset of microalbuminuria, at onset of proteinuria, or in chronic kidney disease (CKD) stage III-IV [95]. This retrospective review demonstrated a delay in kidney replacement therapy by 3 years in the treated CKD group and by 18 years in the treated proteinuric group [95]. These findings were confirmed in a retrospective review of kidney outcomes in men with XLAS from Japan [45]. In this study, men who received ACE inhibitors reached renal failure an average of 22 years later than those who did not receive ACE inhibitors. This beneficial effect of ACE inhibitors was also apparent in the subgroup of men with severe truncating type variants [45]. A randomized, placebo controlled trial of ramipril vs placebo in children with early Alport syndrome (microscopic hematuria alone or microalbuminuria stage) was recently reported [96]. Although not significant due to low enrollment, patients randomized to ramipril had decreased risk of progression of proteinuria and slower decline of GFR compared to patients randomized to placebo [96]. An open-label arm of this study demonstrated no safety concerns in over 200 patient years of treatment with ramipril [96].

Current clinical practice guidelines recommend treatment with an ACE inhibitor for affected males with XLAS and males and females with ARAS at the time of diagnosis if older than 12–24 months. (Table 16.2). Treatment should be started for females with XLAS and males and females with heterozygous variants in COL4A3 or COL4A4 when microalbuminuria is present [97]. Similar to other children with CKD, blood pressures

Table 16.2 Recommendations for timing of treatment with ACE inhibitors in patients with Alport syndrome

Genetic results	Indication for treatment
ARAS or male with XLAS	At time of diagnosis if age >12–24 months
XLAS female	Microalbuminuria
ADAS (heterozygous variant in *COL4A3* or *COL4A4*)	Microalbuminuria

ARAS autosomal recessive Alport syndrome; *XLAS* X-linked Alport syndrome; *ADAS* autosomal dominant Alport syndrome

should be controlled to the 50% for age, gender, and height in children with AS in order to slow the progression of kidney disease [98].

A number of additional agents are currently in clinical development for treatment of Alport syndrome kidney disease. MicroRNAs are small, highly conserved RNAs that regulate gene expression post-transcription. One of these microRNAs, microRNA-21, is upregulated in kidneys of mice with Alport syndrome and contributes to fibrosis [99]. Treatment of Alport mice with an anti-microRNA 21 agent reduces proteinuria and kidney fibrosis and prolongs lifespan [99]. This agent is undergoing testing in a randomized phase II clinical trial in adult patients with Alport syndrome (NCT02855268). Bardoxolone is a second agent currently being tested in a randomized phase II/III clinical trial in patients with Alport syndrome (NCT03019185). Bardoxolone activates Nrf-2 and inhibits NF κB to upregulate the antioxidant response and decrease proinflammatory signaling [100]. In a clinical trial in patients with kidney disease due to type 2 diabetes, bardoxolone increased eGFR; however, the trial was halted due to increased risk of hospitalization and death from heart failure in the bardoxolone treated patients [101]. Bardoxolone treated patients also demonstrated increased proteinuria [101]. It remains controversial whether patients with Alport syndrome will have sustained benefit from treatment with bardoxolone, and long-tern studies will be required to demonstrate value in slowing progression of CKD [102].

Kidney Transplantation

In general, outcomes following kidney transplantation in patients with AS are excellent [103]. 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 ESKD. 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 ESKD in women with AS [46], 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 [104]. One donor had proteinuria prior to transplant and all had microscopic hematuria. Three donors developed new onset hypertension and two developed new proteinuria while kidney function declined by 25–60% over 2–14 years after donation in four of the donors, highlighting the increased donor risk in this population [104].

Overt anti-GBM disease occurs in 3–5% of transplanted AS males [105]. 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 recurrence in subsequent allografts is high. In males with XLAS, the primary target of anti-GBM antibodies is the α5(IV) chain [106, 107]. 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 [106, 108]. The α3(IV) chain is also the target of Goodpasture autoantibodies, but the epit-

ope identified by these antibodies differs from the α3(IV) epitope recognized by ARAS anti-GBM alloantibodies [109].

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

This autosomal dominant disorder results from variants in the *COL4A1* gene (Table 16.1) [9, 110, 111]. Complete absence of *COL4A1* is embryonic lethal in mice [112]. Missense variants that allow for expression of an abnormal α1(IV) chain lead to the development of HANAC syndrome. Kidney findings include gross and microscopic hematuria, cysts and CKD. 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 kidney 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 variants in HANAC syndrome families affect highly conserved glycine residues in the collagenous domain of the α1(IV) chain, potentially affecting integrin binding sites. It is likely that a wider spectrum of disease will emerge in association with both *COL4A1* and *COL4A2* variants as well as variants in other basement membrane genes as larger cohorts of patients with kidney disease phenotypes undergo whole exome and whole genome sequencing [113].

Acknowledgements The authors would like to acknowledge Dr. Clifford Kashtan for his contributions to earlier versions of this chapter.

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