Pediatr Nephrol (2000) 14:502–512

BASIC SCIENCE REVIEW

Clifford E. Kashtan

Alport syndromes: phenotypic heterogeneity of progressive hereditary nephritis

Received: 5 August 1999 / Revised: 25 October 1999 / Accepted: 25 October 1999

Abstract Alport syndrome is a primary genetic disease of basement membranes, manifested clinically as a progressive nephropathy variably associated with sensorineural deafness and a plethora of ocular abnormalities. The long-recognized phenotypic heterogeneity of Alport syndrome may be considered on several levels, including basement membrane biochemistry, basement membrane ultrastructure, the natural history of the nephropathy, and the occurrence of extrarenal abnormalities. This review discusses the possible molecular bases for the heterogeneity. The discussion draws upon recent insights into the molecular genetics of Alport syndrome, and the biochemistry of normal and Alport syndrome basement membranes, in order to provide a framework for understanding the variable renal and extrarenal manifestations of the disease.

Key words Alport syndrome · Molecular genetics · Heterogeneity · Extrarenal disease

Introduction

Alport syndrome is a primary genetic disease of basement membranes, manifested clinically as a progressive nephropathy variably associated with sensorineural deafness and a plethora of ocular abnormalities. It has long been recognized that Alport syndrome is a phenotypically heterogeneous disorder. The phenotypic heterogeneity of Alport syndrome can be considered on several levels, including basement membrane biochemistry, basement membrane ultrastructure, the natural history of the nephropathy, and the occurrence of extrarenal abnormalities. The purpose of this review is to discuss the possible molecular bases for this heterogeneity. This discussion will draw upon recent insights into the molecular genetics of Alport syndrome, and the biochemistry of normal and Alport basement membranes, in order to provide a framework for understanding the variable manifestations of the disease.

Epithelial and endothelial cells rest on structures known as basement membranes. These sheet-like structures are comprised of a collagenous scaffolding consisting primarily of type IV collagen that interacts with other extracellular matrix glycoproteins, such as laminin, entactin, and proteoglycans. The type IV collagen protein family includes six isomeric chains, designated α1(IV)–α6(IV) [1]. Each chain is encoded by a distinct gene, *COL4A1–COL4A6*. The six 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 shared features of type IV collagen α chains include a collagenous domain of about 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 carboxy-terminal noncollagenous (NC1) domain of about 230 residues and a noncollagenous amino-terminal sequence of 15–20 residues. Approximately 20 interruptions of the collagenous triplet sequence are present in the collagenous domain. The NC1 domains each contain 12 completely conserved cysteine residues, which participate in intrachain and interchain disulfide bonds.

Type IV collagen α chains form trimers through associations between their carboxy-terminal NC1 domains, accompanied by folding of the collagenous domains into triple helices [2]. These trimers form networks through several types of intermolecular interaction, including end-to-end linkages between the carboxy-terminal domains of two trimers, covalent interactions between four trimers at their amino-terminal ends, and lateral associations between trimers via binding of the carboxy-terminal domains to sites along the collagenous region of

C.E. Kashtan
University of Minnesota Medical School,
Department of Pediatrics, Division of Pediatric Nephrology,
Minneapolis, Minnesota, USA

C.E. Kashtan (✉)
University of Minnesota Medical School, Box 491,
515 Delaware Street SE, Minneapolis, MN 55455, USA
e-mail: kasht001@maroon.tc.umn.edu
Tel.: +1-612-6249193, Fax: +1-612-6262791

the trimer [3, 4]. Disulfide bonds involving conserved cysteine residues are critical to the interactions between NC1 domains. The various linkages between type IV collagen molecules produce a nonfibrillar polygonal assembly that serves as scaffolding for the deposition of other matrix glycoproteins and for cell attachment.

Molecular genetics of Alport syndrome

Alport syndrome arises from mutations of type IV collagen, the predominant collagenous constituent of basement membranes. Alport syndrome is a genetically heterogeneous disorder (Fig. 1). About 80%–85% of patients have the X-linked form of Alport syndrome, resulting from mutations in the *COL4A5* gene [5]. Most of the remaining patients have autosomal recessive Alport syndrome due to mutations in either *COL4A3* or *COL4A4* [5]. A small minority of families exhibits autosomal dominant disease, which may also arise from mutations in *COL4A3* or *COL4A4* [6].

X-linked Alport syndrome

Several hundred mutations in the *COL4A5* gene causing Alport syndrome have been identified to date [5]. These mutations are distributed throughout the gene, with no apparent hot spots, and with few exceptions each family carries a unique mutation. Approximately 10%–15% of *COL4A5* mutations are de novo, having occurred in the gamete of a parent; 5%–15% of *COL4A5* mutations consist of major rearrangements of the gene, such as large deletions. Other reported types of mutation in *COL4A5* include missense mutations causing amino acid substitutions (approximately 35%), mutations resulting in premature stop codons (nonsense mutations, small frame-shifting deletions, or insertions, splice site mutations – approximately 40%), small in-frame deletions, and splicing mutations that result in skipping of one or more exons. Mutations in the promoter of the *COL4A5* gene have not been reported.

The most-common type of *COL4A5* mutation is a missense substitution involving replacement of a guanine in the first or second position of a glycine codon, resulting in the substitution of a glycine residue in the collagenous domain of α5(IV) by another amino acid. Such mutations are thought to interfere with the normal folding of the mutant α5(IV) chain into triple helices with other type IV collagen α chains. Glycine lacks a side chain, making it the least bulky of amino acids, and is sufficiently small so that three glycine residues can fit into the interior of a tightly wound triple helix. The presence of a bulkier amino acid in a glycine position presumably creates a kink or an unfolding in the triple helix. Glycine substitutions account for a large proportion of disease-causing mutations in collagens I, II, III, IX, X, and XI [7]. Abnormally folded collagen triple helices exhibit increased susceptibility to proteolytic degradation, potentially resulting in the destruction of the mutant chain, as well as the normal chains with which it has formed triple helices. The position of the substituted glycine, or the substituting amino acid itself, may influence the effect of the mutation on triple helical folding, and ultimately the impact of the mutation on the severity of the clinical phenotype.

Rare missense mutations in the *COL4A5* gene involve critical residues in the carboxy-terminal noncollagenous (NC1) domain of the α5(IV) chain, such as 1 of the 12 conserved cysteine moieties. The loss of 1 of these cysteines would eliminate a disulfide bond, which could interfere with the formation of triple helices, or with the construction of networks involving α5(IV) chains.

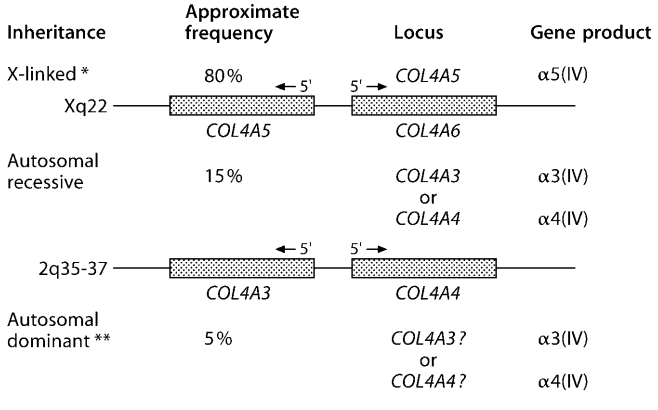

Fig. 1 Genomic organization of *COL4A3–COL4A6* genes, and genetic forms of Alport syndrome. *The Alport syndrome-diffuse leiomyomatosis complex arises from deletions that encompass some portion of the 5'-end of *COL4A5* and terminate within the second intron of *COL4A6*. **Autosomal dominant Alport syndrome (ADAS) has been mapped to 2q35–37 in at least one family [5]. A specific mutation in *COL4A3* or *COL4A4* causing ADAS has yet to be reported. Heterozygous carriers of *COL4A3* and *COL4A4* mutations may have asymptomatic hematuria [9, 12, 13]

Autosomal recessive Alport syndrome

To date, mutations causing autosomal recessive Alport syndrome (ARAS) have been reported in the *COL4A3* gene in 6 patients [8–11], and in the *COL4A4* gene in 12 patients [11, 12]. Some of these patients are homozygous for their mutations, some are compound heterozygotes, and others are heterozygotes in whom only one of the mutant alleles has been identified. Reported *COL4A3* and *COL4A4* mutations include nonsense, frame-shift, splicing, and missense alterations. As with *COL4A5*, there appear to be no mutation hot spots in *COL4A3* or *COL4A4*. Individuals who are heterozygous for *COL4A3* or *COL4A4* mutations may display hematuria [9, 12], and mutations of *COL4A4* have been implicated in autosomal dominant benign familial hematuria [13].

Autosomal dominant Alport syndrome

Autosomal dominant Alport syndrome (ADAS) has been mapped in a single family to chromosome 2, in proximi-

ty to *COL4A3* and *COL4A4* [6]. A specific mutation of either gene in this family has yet to be reported.

Heterogeneity of the Alport phenotype

Heterogeneity of basement membrane biochemistry

The availability of monospecific antibodies against each of the six type IV collagen α chains has made it possible to characterize the changes in type IV collagen expression that occur as a result of mutations in the *COL4A3*, *COL4A4*, or *COL4A5* genes [14–20]. These changes are diagnostically useful and have been extensively illustrated in recent reviews [17, 21, 22].

Normally, the α3(IV), α4(IV), and α5(IV) chains are highly expressed in basement membranes that are definitively, or in some cases possibly, involved in Alport syndrome: glomerular basement membrane (GBM), anterior lens capsule, Descemet's membrane, Bruch's membrane, and several basement membranes of the cochlea, including the basilar membrane and the basement membranes of the stria vascularis, spiral limbus, and spiral prominence [15, 23–30]. In 70%–80% of males with X-linked Alport syndrome (XLAS), the GBM, distal tubular basement membrane (TBM), and Bowman's capsules fail to stain for the α3(IV), α4(IV), and α5(IV) chains, but expression of the α1(IV) and α2(IV) chains is preserved and is increased [18, 31]. Basement membranes of some males with XLAS exhibit normal, or reduced but positive, staining for the α3(IV), α4(IV), and α5(IV) chains [16]. The α6(IV) chain is not expressed in Bowman's capsule or distal TBM of XLAS males whose basement membranes lack α5(IV) expression. Epidermal basement membranes (EBM) normally express the α1(IV), α2(IV), α5(IV), and α6(IV) chains, but not the α3(IV) or α4(IV) chains. Most males with XLAS show no EBM expression of α5(IV) or α6(IV). Lens capsules of some males with XLAS do not express the α3(IV), α4(IV, or α5(IV) chains, while expression of these chains appears normal in other patients [32]. The expression of type IV collagen chains in the cochleae of human Alport patients has not been studied.

Women who are heterozygous for XLAS mutations frequently exhibit mosaicism of GBM and distal TBM expression of the α3(IV), α4(IV), and α5(IV) chains, while expression of the α1(IV) and α2(IV) chains is preserved [16, 21, 26]. Female heterozygotes frequently also display mosaicism of EBM staining for α5(IV).

In patients with ARAS, GBMs usually show no expression of the α3(IV), α4(IV), or α5(IV) chains, but α5(IV) and α6(IV) are strongly expressed in Bowman's capsule, distal TBM, and EBM [20, 33, 34]. Therefore, XLAS and ARAS may be distinguished by immunohistochemical analysis of renal biopsy specimens. It should be noted that occasional patients with *COL4A5* mutations may show weak expression of α5(IV) and α6(IV) in Bowman's capsule or distal TBM, and no expression of the α3(IV), α4(IV), or α5(IV) chains in GBM [35]. As in XLAS, the absence of α3(IV), α4(IV), and α5(IV) chains from GBM is associated with a marked increase in the expression of α1(IV) and α2(IV) chains. The expression of type IV collagen chains in basement membranes of patients with ADAS has not been studied, but is likely to be normal.

These observations, along with immunochemical studies of extracted normal and Alport basement membranes [36–38], strongly suggest that basement membranes contain several distinct type IV collagen networks. Normal GBM appears to contain at least two networks: a major network composed of α3(IV), α4(IV), and α5(IV) chains, and a minor network consisting of the α1(IV) and α2(IV) chains. In Bowman's capsule, distal TBM, and EBM the α6(IV) chain is available as a partner for the α5(IV) chain, explaining why in ARAS patients the α5(IV) chain persists in these basement membranes but is absent from GBM.

The abnormalities of type IV collagen expression observed in XLAS and ARAS patients indicate that a mutation affecting one of the chains involved in the putative α3-α4-α5(IV) network can prevent basement membrane expression not only of that chain but of the other two chains as well. Similarly, a mutation involving the α5(IV) chain can interfere with basement membrane expression of α6(IV). The mechanisms that produce these effects remain under investigation. It is likely that at least some mutations interfere with the formation of trimeric type IV collagen molecules, leading to degradation of normal chains that have been prevented from forming trimers, or that have formed abnormal trimers. This kind of process accounts for abnormal type I collagen deposition in bone in osteogenesis imperfecta [39]. In some instances, a mutation at one of the type IV collagen loci may result in reduced transcription of other type IV collagen genes, or may accelerate degradation of mRNA transcribed from these genes. Thorner et al. [40] found that kidneys of male dogs with Samoyed hereditary nephropathy, a canine form of Alport syndrome that arises from a *COL4A5* mutation, contained levels of mRNA for the α3(IV) and α4(IV) chains that were substantially less than in unaffected males. Thus far, this observation has not been supported by other studies. Nakanishi et al. [41] found no differences in mRNA levels for α3(IV) and α4(IV) chains, measured by competitive reverse transcription-polymerase chain reaction, in kidneys of men with XLAS compared with normal male kidneys. Sasaki et al. [42] observed that dermal fibroblasts of males with XLAS express normal levels of α6(IV) mRNA, despite the absence of α6(IV) protein in their EBMs. In transgenic mice with *COL4A3* mutations, renal mRNA levels for the α4(IV) and α5(IV) chains are not different from levels in normal mice [43, 44]. The results of these studies suggest that post-transcriptional events account for the ability of a mutation affecting one of the type IV collagen genes to prevent tissue expression of normal type IV collagen chains.

There is evidence to suggest that *COL4A5* mutations that are associated with preservation of GBM expression

Table 1 Hypothetical relationships between the mutation type, basement membrane (*BM*) expression of the α3-α4-α5(IV) network, and clinical phenotype (*ESRD* end-stage renal disease)

Type of *COL4A5* mutation	Deletion, nonsense	Missense	Splicing (reading frame maintained), some missense mutations
	↓	↓	↓
Effect on triple helices involving α5(IV)	Triple helices involving α5(IV) do not form	Triple helices involving α5(IV) fold abnormally and undergo rapid degradation	Triple helices involving α5(IV) fold abnormally but degradation is slow
	↓	↓	↓
Effect on α3-α4-α5 network	α3-α4-α5 network is absent from BM	α3-α4-α5 network is absent from BM	α3-α4-α5 network present in BM in reduced amount, or forms abnormal networks
	↓	↓	↓
Phenotype	Juvenile ESRD, deafness	Juvenile ESRD, deafness	Adult ESRD, hearing may be normal or deteriorate slowly

of α3(IV), α4(IV), and α5(IV) chains may result in a less-severe phenotype [16, 35, 45]. Information about the effects of individual *COL4A5* mutations on basement membrane expression of these chains, and the relationship of chain expression to phenotype, is still being accumulated; hypothetical relationships between the mutation type, basement membrane expression of the α3-α4-α5(IV) network, and clinical phenotype are presented in Table 1. Naito et al. [35] observed absence of renal basement membrane α3(IV), α4(IV), and α5(IV) chains in two males with *COL4A5* deletions, but these chains were detectable in four of six males with glycine substitutions in α5(IV). Knebelmann et al. [46] observed absence of these chains from renal basement membranes in eight of nine males with glycine substitutions in α5(IV).

Heterogeneity of basement membrane ultrastructure

The pathognomonic ultrastructural feature of the Alport kidney is a thickened GBM, in which the lamina densa has been transformed into a heterogeneous network of interwoven lamellae enclosing clear electron-lucent areas; these may contain round granules of variable density measuring 20–90 nm in diameter [47–49]. These changes can be seen regardless of inheritance pattern or the presence or absence of sensorineural deafness or ocular abnormalities.

This lesion is found in most, but not all, patients with Alport syndrome. Affected young males, heterozygous females at any age, and affected adult males on occasion, may have diffusely attenuated GBM measuring 100 nm or less, rather than the pathognomonic lesion. Studies of males with human or canine Alport syndrome have shown that the earliest manifestation of the GBM lesion is attenuation, and that the extent and severity of thickening and multilamellation increase with age [50, 51]. Heterozygous females may have GBM of normal appearance or, at the other end of the spectrum, diffuse GBM thickening and multilamellation, but most will display a mixture of normal, thin and thick, lamellated GBM [52].

Although diffuse attenuation of GBM has been considered the hallmark of benign familial hematuria, some patients with GBM attenutation have progressive renal disease and a *COL4A5* mutation [46, 53]. These cases illustrate the fact that "thin GBM" is a descriptive term, rather than a specific disease entity.

The influence of gender on the expression of the Alport phenotype

About 80% of people with Alport syndrome have the X-linked form of the disease. As expected for an X-linked condition, all affected males exhibit the disease phenotype, although the natural history of the renal, cochlear, and ocular aspects of the disease varies from family to family. Thus, it can be predicted that an affected male in an XLAS kindred will inevitably develop kidney failure at some time in his life and, if deafness is a feature of the phenotype in that kindred, that he will eventually exhibit hearing loss. In contrast, affected females in XLAS kindreds typically display mild clinical involvement, particularly during childhood and young adulthood. At least 90% of heterozygous females exhibit microhematuria. However, the incidence of chronic renal failure before 40–50 years of age is probably no greater than 10%–15% in heterozygous females.

The lesser severity of disease in heterozygous females most likely reflects the presence of a normal *COL4A5* allele. A random pattern of X chromosome inactivation would be expected to result, in most heterozygotes, in an approximate 1:1 ratio between cells expressing the normal *COL4A5* allele and cells expressing the mutant allele. A small percentage of heterozygotes would lie at either end of the spectrum, with predominant inactivation of either the normal or the mutant *COL4A5* allele. Results of studies of the skin basement membrane expression of the α5(IV) chain in females who are heterozygous for XLAS support these predictions. The basement membrane underlying the epidermis (EBM) normally expresses the α5(IV) chain, but in most males with XLAS, EBM expression of the α5(IV) chain is completely lacking [14, 17]. Most heterozygous females exhibit a mosaic pattern of α5(IV) expression in the EBM [16]. In a study of 25 females heterozygous for XLAS, Nakanishi et al. [54] determined an α5(IV) expression ratio, i.e., the fraction of total EBM expressing the α5(IV) chain.

The mean (SD) ratio was 56% (27%), with a range of 8%–94%, and the ratios were normally distributed. We have obtained similar results in female dogs that are heterozygous for XLAS [55]: the mean α5(IV) expression ratio in EBM was 61% (16%), with a range of 21%–100%, and the ratios were normally distributed (C. Kashtan, G. Lees, unpublished observations). These results are consistent with the results one would predict on the basis of random X inactivation in basement membrane-producing cells of the epidermis.

While we intuitively would predict that heterozygous females with renal insufficiency would exhibit skewed inactivation patterns favoring the X chromosome carrying the mutant *COL4A5* allele, this relationship has not been clearly established. Guo et al. [56] described an XLAS female with a severe phenotype in whom 90% of the *COL4A5* mRNA in white blood cells and kidney was derived from the mutant allele. Vetrie et al. [57], studying X inactivation patterns in white blood cells of XLAS females, found a symmetrical pattern in most subjects and occasional subjects with extreme skewing of inactivation. However, these authors were unable to demonstrate a correlation between the X inactivation pattern and disease severity in females with XLAS [57]. While Nakanishi et al. [54] found an inverse correlation between the EBM α5(IV) expression ratio, an indirect indicator of the X inactivation pattern, and urinary protein excretion in heterozygous females , we have been unable to confirm this relationship in a dog model of XLAS (C. Kashtan, G. Lees, unpublished observations).

These various observations support the notion that the presence in heterozygous females of a normal *COL4A5* allele, and consequently some level of α5(IV) expression in basement membranes, is indeed protective of renal function. However, it is not yet clear whether or not a threshold protective level of α5(IV) expression exists, or what this threshold level might be. Although there is currently no evidence that modifying genes affect the natural history of Alport syndrome in affected males, this issue has yet to be addressed in heterozygous females.

While some heterozygous females with severe manifestations of XLAS would be expected simply on the basis of random X inactivation, nonrandom events favoring expression of the mutant *COL4A5* allele may also occur. For example, Kapoor and Dasgupta [58] described a 20-year-old woman with anterior lenticonus and other features of a severe Alport phenotype, who had a 45,XO karyotype.

Heterogeneity of the natural history of the Alport nephropathy

Native kidney disease

End-stage renal disease (ESRD) develops in virtually all affected males with XLAS, but the rate of progression shows significant interkindred variability. A bimodal distribution of age at ESRD has been observed among families with XLAS [59]. In families with so-called juvenile Alport syndrome, the mean age at ESRD in affected males is less than 31 years, while in adult Alport syndrome the mean age at ESRD is greater than 31 years. Because the standard deviation around the mean age at ESRD is about 5–7 years, the timing of ESRD in an affected male can be estimated if information about related affected males is available. However, marked intrakindred variability in the rate of progression to renal failure of affected males has occasionally been reported in families with missense *COL4A5* mutations [60].

Compared with affected males, the renal prognosis in affected females with XLAS is generally benign, with most surviving into old age with clinically mild renal disease. Approximately 15% of female heterozygotes develop renal insufficiency during adolescence, young adulthood, or middle age. Many women with progressive nephritis maintain adequate renal function until late in life; the true incidence of renal failure in elderly heterozygotes remains to be determined. Gross hematuria in childhood, nephrotic syndrome, and diffuse GBM thickening by electron microscopy are features suggestive of a progressive course in affected females [61]. Sensorineural deafness and anterior lenticonus are also indicative of an unfavorable renal outcome in affected women.

Male patients with *COL4A5* mutations that result in an absent or truncated α5(IV) chain, such as deletions and nonsense mutations, consistently exhibit juvenile ESRD, usually associated with deafness [5, 53]. Most of the missense and splicing mutations of *COL4A5* described thus far are also associated with juvenile ESRD and deafness. Several missense mutations of *COL4A5* have been associated with adult ESRD and late development of deafness [5, 53, 62]. With regard to missense mutations affecting glycine residues in the collagenous domain of the α5(IV) chain, there is at present no clear correlation between the position of the substituted glycine, or the substituting amino acid, and the resulting clinical phenotype. Splicing mutations that cause skipping of an exon but maintain the *COL4A5* reading frame may also be associated with adult ESRD [46].

Patients with ARAS consistently exhibit juvenile ESRD, regardless of gender [8, 10–12, 63]. Patients with ADAS appear to exhibit a slower rate of progression to ESRD than most patients with XLAS [64].

With regard to the progressive nature of the Alport nephropathy, it is worth noting that a mutation in the *COL4A3*, *COL4A4*, or *COL4A5* gene results in renal failure indirectly. In the normal developing kidney, α1 (IV) and α2(IV) chains predominate in the primordial GBM of immature glomeruli [30, 31, 65]. The formation of capillary loops within the maturing glomeruli is associated with the appearance of α3, α4, and α5(IV) chains in the GBM. As glomerular maturation progresses, the α3, α4, and α5(IV) chains become the predominant type IV collagen chains in GBM. This process has been referred to as “isotype switching” [30]. Although this isotype switch does not occur in Alport syndrome [30, 31, 65], glomerular development otherwise pro-

ceeds normally, and the GBM of young animals and children with Alport syndrome exhibits a normal trilaminar appearance by electron microscopy [43, 44, 50, 51]. These glomeruli exhibit normal capacities for filtration and for selective permeability, as demonstrated by the normal glomerular filtration rates and absence of overt proteinuria that are characteristic of early Alport syndrome in both humans and animals. Hearing is likewise normal early in life, with deafness becoming apparent and progressing with increasing age. Therefore it appears that proteinuria and renal insufficiency, as well as sensorineural deafness, result from processes that are initiated by the absence of the α3-α4-α5(IV) network, rather than arising directly from the absence of this network. These processes probably include, but may not be limited to, overexpression in the GBM of the αl(IV) and α2(IV) chains, type V collagen, and type VI collagen [18, 20, 31, 44, 65–67].

Although most research on Alport syndrome focuses on the glomerulus, it is important to note that, as in other chronic glomerulopathies, expansion of the renal interstitial compartment shows a strong inverse correlation with glomerular filtration rate in males with Alport syndrome [68]. It is possible that independent genetic, dietary, or environmental factors influence the rapidity of renal interstitial expansion that occurs as a downstream effect of *COL4A3*, *COL4A4*, and *COL4A5* mutations. In this regard, the provocative report of Callis et al. [69], describing diminished proteinuria and stabilization of renal function in a small group of Alport males treated chronically with cyclosporine, raises several questions. First, of course, is the question of whether these effects can be replicated in other people and in animals with Alport syndrome. Other questions concern the mechanism of the effect of cyclosporine on protein excretion in Alport patients, and whether stabilization of renal function is a consequence of the reduced proteinuria or an independent effect of cyclosporine.

Transplant outcome

At present, renal transplantation is the only available treatment for Alport syndrome. The data of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) document equivalent allograft survival rates in patients with familial nephritis and patients with other diagnoses [70]. Anti-GBM nephritis involving the renal allograft is a rare, but dramatic, manifestation of Alport syndrome, occurring in 3%–4% of transplanted male Alport patients [71–75]. Alport patients who develop post-transplant anti-GBM nephritis are usually male, always deaf, and likely to have reached ESRD before age 30 years [22]. This profile describes the majority of Alport patients presenting for renal transplantation, limiting its predictive value. However, Alport patients with normal hearing or late progression to ESRD are at very low risk for the development of post-transplant anti-GBM nephritis. Females with XLAS also appear to at low risk for this complication.

The pathogenesis of post-transplant anti-GBM nephritis in Alport syndrome patients is presumably based upon exposure to antigens present in the donor GBM, for which the recipient has not established immune tolerance [76]. The target(s) of anti-GBM antibodies in some of these patients has been determined, with variable results. Most of those with XLAS exhibit antibodies against the NC1 domain of the α5(IV) chain, but antibodies against α3(IV) NC1 have also been described [77–79]. In ARAS patients with anti-GBM nephritis, antibodies appear to target the α3(IV) NC1 domain [77].

Females who are heterozygous for *COL4A5* mutations would not be expected to be at risk for the development of post-transplant anti-GBM nephritis, since the product of the normal *COL4A5* allele would allow establishment of immunological tolerance for α5(IV). Nevertheless, post-transplant anti-GBM nephritis has been reported in two females with Alport syndrome, both of whom proved to have ARAS, due to *COL4A3* mutations [8, 10, 80].

There is evidence to suggest that mutations in the *COL4A5* gene that prevent expression of an immunogenic gene product, thereby preventing the establishment of tolerance for α5(IV), are associated with an increased risk for the development of post-transplant anti-GBM nephritis [81]. For example, a recent review of the genetics of Alport syndrome included seven patients with XLAS and post-transplant anti-GBM nephritis; six had large deletions of *COL4A5* and the other had a splicing mutation [5]. Even if certain types of *COL4A5* mutation confer a higher risk of developing post-transplant anti-GBM nephritis, such data are currently of limited value in planning transplantation. It is clear that Alport patients with *COL4A5* deletions can undergo renal transplantation without developing anti-GBM nephritis, indicating that other factors, presently unknown, must influence the initiation and elaboration of the immune response to the allograft [5, 82]. Patients with ARAS can also be transplanted successfully [83].

Heterogeneity of extrarenal disease in Alport syndrome

Leiomyomatosis

The association of Alport syndrome with leiomyomatosis of the esophagus and tracheobronchial tree has been reported in approximately 20 families [84]. Affected females in these kindreds typically also exhibit genital leiomyomas, causing clitoral hypertrophy with variable involvement of the labia majora and uterus. Bilateral posterior subcapsular cataracts also occur frequently in affected individuals in these kindreds. Symptoms usually appear in late childhood and include dysphagia, postprandial vomiting, retrosternal or epigastric pain, recurrent bronchitis, dyspnea, cough, and stridor.

All families in which XLAS cosegregates with diffuse leiomyomatosis exhibit large deletions that span the adjacent 5’ ends of the *COL4A5* and *COL4A6* genes [85,

86]. The deletions involve varying lengths of *COL4A5*, but the *COL4A6* breakpoint is always located in the second intron of the gene [87, 88]. These deletions appear to arise as a result of recombination events involving repetitive LINE-1 elements, at least in some cases [89, 90]. Leiomyomatosis does not occur in patients with deletions of *COL4A5* and *COL4A6* that extend beyond intron 2 of *COL4A6*. Mutations of *COL4A6* alone do not appear to cause Alport syndrome, consistent with the absence of the α6(IV) chain from normal GBM [86, 91]. The fact that leiomyomatosis is so rare among patients with XLAS, most of whom lack tissue expression of the α6(lV) chain, indicates that these tumors do not develop simply from absence of the α6(IV) chain in smooth muscle. The pathogenesis of these tumors remains under investigation.

Deafness

High-frequency, sensorineural hearing loss is frequently but not universally associated with the Alport renal lesion, occurring in an estimated 55% of males and 45% of females with the disease [92]. The true incidence of deafness may be higher, since patients are not always evaluated with audiograms, and the prevalence of deafness increases with age. Males with XLAS exhibit deafness earlier in life, and develop more severe hearing deficits, than heterozygous females. There are no gender differences in the occurrence or progression of deafness in ARAS. In some families with XLAS and apparently normal hearing, deafness may be a late and very slowly progressive phenomenon [62].

Male patients with *COL4A5* mutations that result in an absent or truncated α5(IV) chain, such as deletions and nonsense mutations, usually exhibit deafness that is detectable in the first 2 decades of life [53]. Most of the missense and splicing mutations of *COL4A5* described thus far are also associated with juvenile onset of deafness. Several missense mutations of *COL4A5* that are associated with adult ESRD are also characterized by late development of deafness [5, 62].

Ocular abnormalities

Ocular defects are common in patients with Alport syndrome. The types of ocular lesions exhibited by XLAS and ARAS patients are similar [93, 94]. Anterior lenticonus, in which the central portion of the lens protrudes into the anterior chamber, is pathognomonic of Alport syndrome [95], and appears to be almost entirely restricted to Alport families exhibiting juvenile-type nephropathy and deafness [59]. In XLAS families anterior lenticonus is far more common in affected males, but can occur in females. Anterior lenticonus appears to arise as a result of mechanical weakness of the anterior lens capsule, which exhibits attenuation and fracturing by light and electron microscopy [96–98]. This fragility of the anterior lens capsule presumably reflects absence or deficiency of the α3-α4-α5(IV) network [32].

A variety of other ocular lesions have been reported in patients with Alport syndrome. The most-common abnormalities are pigmentary changes in the perimacular region, consisting of whitish or yellowish granulations, or flecks, surrounding the foveal area [99–101]. These lesions are frequently accompanied by anterior lenticonus, but may occur in the absence of lenticonus. The occurrence of flecks is correlated with deafness, and the incidence appears to increase with age [99, 102]. Retinal flecks may represent abnormalities of Bruch's membrane, the basement membrane supporting the retinal pigment epithelium.

Corneal endothelial vesicles (posterior polymorphous dystrophy) observed in some Alport patients [103–105] may arise from defects in Descemet's membrane, the basement membrane underlying the corneal endothelium. Recurrent corneal erosion in Alport patients has been attributed to alterations of the corneal epithelial basement membrane [106, 107]. At present there do not appear to be clear correlations between the type of *COL4A5* mutation and the occurrence or nature of ocular abnormalities in individuals with XLAS [102].

Mental retardation

Jonsson et al. [108] recently described the co-occurrence of Alport syndrome, *m*ental retardation, *m*idface hypoplasia, and *e*lliptocytosis in two brothers (AMME complex), who were shown to have a microdeletion involving the entire *COL4A5* gene, and extending beyond its 3' end. To date, two new genes have been identified in the deleted region downstream of the *COL4A5* gene: *FACL4*, which encodes a long-chain acyl-CoA synthetase, and *AMMECR1*, a gene that is evolutionarily conserved and encodes a protein with as yet unknown functions [109, 110]. The association of hereditary nephritis with mental retardation is a rare occurrence [111].

Hematological abnormalities

In 1972, a family was described in which hereditary nephritis segregated with deafness and megathrombocytopenia, and reports of several similar families have subsequently appeared [112–118]. In most of the families described, the disorder appears to have been transmitted as an autosomal dominant trait. GBM thickening and lamellation were found in two of the reported patients.

The association of these platelet defects, and in some families granulocyte abnormalities [119], with hereditary nephritis remains unexplained. There have been few reports of gene localization or mutation analysis in these families, except for negative reports regarding *COL4A5* involvement, so the genetic locus or loci involved remains unknown. Basement membranes of these patients show normal expression of type IV collagen α chains

[120]. As noted above, the association of Alport syndrome and elliptocytosis has been described in a single family [108].

Summary

It is now evident that the phenotypic features of Alport syndrome arise from mutations in the *COL4A3*, *COL4A4*, or *COL4A5* genes that interfere with the incorporation into basement membranes of a type IV collagen network composed of α3(IV), α4(IV), and α5(IV) chains. Absence or deficiency of this network triggers molecular processes that gradually produce severe alterations in the architecture and function of the basement membranes of the glomerulus, cochlea, and eye, resulting in progressive glomerulopathy, high-frequency sensorineural deafness, and acquired ocular anomalies. Certain correlations between mutation and phenotype have been established, such as the precise nature of the deletion mutations associated with the Alport syndrome/diffuse leiomyomatosis complex, and the consistent association of deletion and nonsense mutations with the development of ESRD and deafness during the first several decades of life, at least in affected males. The rules governing the phenotypic consequences of missense *COLA5* mutations have yet to be determined. Also, it is not yet possible to define a simple relationship between X inactivation patterns and the outcome of females who are heterozygous for *COL4A5* mutations. Ongoing efforts to correlate genotype and phenotype in Alport patients, and to elucidate the pathogenesis of chronic renal failure and deafness, should eventually refine our understanding of the factors that determine the fate of an individual with a mutation in the *COL4A3*, *COL4A4*, or *COL4A5* gene.

References

1. Zhou J, Reeders ST (1996) The a chains of type IV collagen. Contrib Nephrol 117:80–105
2. Dolz R, Engel J, Kuhn K (1988) Folding of collagen IV. Eur J Biochem 178:357–366
3. Timpl R, Wiedemann H, Delden V van, Furthmayr H, Kuhn K (1981) A network model for the organization of type IV collagen molecules in basement membranes. Eur J Biochem 120: 203–211
4. Yurchenco PD, Tsilibary EC, Charonis AS, Furthmayr H (1986) Models for the self-assembly of basement membrane. J Histochem Cytochem 34:93–102
5. Lemmink HH, Schröder CH, Monnens LAH, Smeets HJM (1997) The clinical spectrum of type IV collagen mutations. Hum Mutat 9:477–499
6. Jefferson JA, Lemmink HH, Hughes AE, Hill CM, Smeets HJ, Doherty CC, Maxwell AP (1997) Autosomal dominant Alport syndrome linked to the type IV collagen alpha 3 and alpha 4 genes (*COL4A3* and *COL4A4*). Nephrol Dial Transplant 12: 1595–1599
7. Kuvaniemi H, Tromp G, Prockop DJ (1997) Mutations in fibrillar collagens (types I, II, III, and XI), fibril-associated collagen (type IV), and network-forming collagen (type X) cause a spectrum of diseases of bone, cartilage, and blood vessels. Hum Mutat 9:300–315
8. Ding J, Stitzel J, Berry P, Hawkins E, Kashtan C (1995) Autosomal recessive Alport syndrome: mutation in the *COL4A3* gene in a woman with Alport syndrome and posttransplant antiglomerular basement membrane nephritis. J Am Soc Nephrol 5:1714–1717
9. Knebelmann B, Forestier L, Drouot L, Quinones S, Chuet C, Benessy F, Saus J, Antignac C (1995) Splice-mediated insertion of an *Alu* sequence in the *COL4A3* mRNA causing autosomal recessive Alport syndrome. Hum Mol Genet 4:675–679
10. Lemmink HH, Mochizuki T, Heuvel LPWJ van den, Schroder CH, Barrientos A, Monnens LAH, Oost BA van, Brunner HG, Reeders ST, Smeets HJM (1994) Mutations in the type IV collagen α3 (*COL4A3*) gene in autosomal recessive Alport syndrome. Hum Mol Genet 3:1269–1273
11. Mochizuki T, Lemmink HH, Mariyama M, Antignac C, Gubler MC, Pirson Y, Verellen-Dumoulin C, Chan B, Schroeder CH, Smeets HJM, Reeders ST (1994) Identification of mutations in the α3(IV) and α4(IV) collagen genes in autosomal recessive Alport syndrome. Nat Genet 8:77–82
12. Boye E, Mollet GFL, Cohen-Solal L, Heidet L, Cochat P, Grunfeld J-P, Palcoux J-B, Gubler M-C, Antignac C (1998) Determination of the genomic structure of the *COL4A4* gene and of novel mutations causing autosomal recessive Alport syndrome. Am J Hum Genet 63:1329–1340
13. Lemmink HH, Nillesen WN, Mochizuki T, Schröder CH, Brunner HG, Oost BA van, Monnens LAH, Smeets HJM (1996) Benign familial hematuria due to mutation of the type IV collagen α4 gene. J Clin Invest 98:1114–1118
14. Yoshioka K, Hino S, Takemura T, Maki S, Wieslander J, Takekoshi Y, Makino H, Kagawa M, Sado Y, Kashtan CE (1994) Type IV collagen α5 chain: normal distribution and abnormalities in X-linked Alport syndrome revealed by monoclonal antibody. Am J Pathol 144:986–996
15. Peissel B, Geng L, Kalluri R, Kashtan C, Rennke HG, Gallo GR, Yoshioka K, Sun MJ, Hudson BG, Neilson EG, Zhou J (1995) Comparative distribution of the α1(IV), α5(IV) and α6(IV) collagen chains in normal human adult and fetal tissues and in kidneys from X-linked Alport syndrome patients. J Clin Invest 96:1948–1957
16. Nakanishi K, Yoshikawa N, Iijima K, Kitagawa K, Nakamura H, Ito H, Yoshioka K, Kagawa M, Sado Y (1994) Immunohistochemical study of α1–5 chains of type IV collagen in hereditary nephritis. Kidney Int 46:1413–1421
17. Kashtan CE, Kleppel MM, Gubler MC (1996) Immunohistologic findings in Alport syndrome. Contrib Nephrol 117:142–153
18. Kashtan CE, Kim Y (1992) Distribution of the α1 and α2 chains of collagen IV and of collagens V and VI in Alport syndrome. Kidney Int 42:115–126
19. Kagawa M, Kishiro Y, Naito I, Nemoto T, Nakanishi H, Ninomiya Y, Sado Y (1997) Epitope-defined monoclonal antibodies against type-IV collagen for diagnosis of Alport's syndrome. Nephrol Dial Transplant 12:1238–1241
20. Gubler MC, Knebelmann B, Beziau A, Broyer M, Pirson Y, Haddourn F, Kleppel MM, Antignac C (1995) Autosomal recessive Alport syndrome: immunohistochemical study of type IV collagen chain distribution. Kidney Int 47:1142–1147
21. Kashtan CE (1998) Alport syndrome and thin glomerular basement membrane disease. J Am Soc Nephrol 9:1736–1750
22. Kashtan CE, Michael AF (1996) Perspectives in clinical nephrology: Alport syndrome. Kidney Int 50:1445–1463
23. Butkowski RJ, Wieslander J, Kleppel M, Michael AF, Fish AJ (1989) Basement membrane collagen in the kidney: regional localization of novel chains related to collagen IV. Kidney Int 35:1195–1202
24. Cosgrove D, Kornak JM, Samuelson G (1996) Expression of basement membrane type IV collagen chains during postnatal development in the murine cochlea. Hear Res 100:21–32
25. Kalluri R, Gattone VH, Hudson BG (1998) Identification and localization of type IV collagen chains in the inner ear cochlea. Connect Tissue Res 37:143–150

26. Kleppel MM, Kashtan C, Sand PA, Wieslander J, Michael AF (1989) Distribution of familial nephritis antigen in normal tissue and renal basement membranes of patients with homozygous and heterozygous Alport familial nephritis: relationship of familial nephritis and Goodpasture antigens to novel collagen chains and type IV collagen. Lab Invest 61:278–289
27. Kleppel MM, Michael AF (1990) Expression of novel basement membrane components in the developing human kidney and eye. Am J Anat 187:165–174
28. Kleppel MM, Santi PA, Cameron JD, Wieslander J, Michael AF (1989) Human tissue distribution of novel basement membrane collagen. Am J Pathol 134:813–825
29. Ninomiya Y, Kagawa M, Iyama K, Naito 1, Kishiro Y, Seyer JM, Sugimoto M, Oohashi T, Sado Y (1995) Differential expression of two basement membrane collagen genes, *COL4A6* and *COL4A5*, demonstrated by immunofluorescence staining using peptide-specific monoclonal antibodies. J Cell Biol 130: 1219–1229
30. Miner JH, Sanes JR (1994) Collagen IV α3, α4 and α5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches. J Cell Biol 127: 879–891
31. Kalluri R, Shield CF, Todd P, Hudson BG, Neilson EG (1997) Isoform switching of type IV collagen is developmentally arrested in X-linked Alport syndrome leading to increased susceptibility of renal basement membranes to endoproteolysis. J Clin Invest 99:2470–2478
32. Cheong HI, Kashtan CE, Kim Y, Kleppel MM, Michael AF (1994) Immunohistologic studies of type IV collagen in anterior lens capsules of patients with Alport syndrome. Lab Invest 70:553–557
33. Nomura S, Naito I, Fukushima T, Tokura T, Kataoka N, Tanaka I, Tanaka H, Osawa G (1998) Molecular genetic and immunohistochemical study of autosomal recessive Alport's syndrome. Am J Kidney Dis 31:E4.1
34. Lees G, Helman RG, Kashtan CE, Michael AF, Homco LD, Millichamp NJ, Ninomiya Y, Sado Y, Kim Y (1998) A model of autosomal recessive Alport syndrome in English cocker spaniel dogs. Kidney Int 54:706–719
35. Naito I, Kawai S, Nomura S, Sado Y, Osawa G (1996) Relationship between *COL4A5* gene mutation and distribution of type IV collagen in male X-linked Alport syndrome. Kidney Int 50:304–311
36. Kleppel MM, Kashtan CE, Butkowski RJ, Fish AJ, Michael AF (1987) Alport familial nephritis: absence of 28 kilodalton non-collagenous monomers of type IV collagen in glomerular basement membrane. J Clin Invest 80:263–266
37. Kleppel MM, Fan WW, Cheong HI, Michael AF (1992) Evidence for separate networks of classical and novel basement membrane collagen: characterization of α3(IV)-Alport antigen heterodimers. J Biol Chem 267:4137–4142
38. Gunwar S, Ballester F, Noelken M, Sado Y, Ninomiya Y, Hudson B (1998) 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 273: 8767–8775
39. Prockop DJ (1992) Mutations in collagen genes as a cause of connective-tissue diseases. N Engl J Med 326:540–546
40. Thorner PS, Zheng K, Kalluri R, Jacobs R, Hudson BG (1996) Coordinate gene expression of the α3, α4 and α5 chains of collagen type IV: evidence from a canine model of X-linked nephritis with a *COL4A5* gene mutation. J Biol Chem 271: 13821–13828
41. Nakanishi K, Yoshikawa N, Iijima K, Nakamura H (1996) Expression of type IV collagen α3 and α4 chain mRNA in X-linked Alport syndrome. J Am Soc Nephrol 7:938–945
42. Sasaki S, Zhou B, Fan WW, Kim Y, Barker DF, Derision JC, Atkin CL, Gregory MC, Zhou J, Segal Y, Sado Y, Ninomiya Y, Michael AF, Kashtan CE (1998) Expression of mRNA for type IV collagen α1, α5 and α6 chains by cultured dermal fibroblasts from patients with X-linked Alport syndrome. Matrix Biol 17:279–291
43. Miner JJ, Sanes JR (1996) Molecular and functional defects in kidneys of mice lacking collagen α3(IV): implications for Alport syndrome. J Cell Biol 135:1403–1413
44. Cosgrove D, Meehan DT, Grunkerneyer JA, Kornak JM, Sayers R, Hunter WJ, Samuelson GC (1996) Collagen COL4A3 knockout: a mouse model for autosomal Alport syndrome. Genes Dev 10:2981–2992
45. Mazzucco G, Barsotti P, Muda AO, Fortunato M, Mihatsch M, Torri-Tarelli L, Renieri A, Faraggiana T, De Marchi M, Monga G (1998) Ultrastructural and immunohistochemical findings in Alport's syndrome: a study of 108 patients from 97 Italian families with particular emphasis on *COL4A5* gene mutation correlations. J Am Soc Nephrol 9:1023–1031
46. Knebelmann B, Breillat C, Forestier L, Arrondel C, Jacassier D, Giatras J, Druout L, Deschennes G, Grunfeld JP, Broyer M, Gubler MC, Antignac C (1996) Spectrum of mutations in the *COL4A5* collagen gene in X-linked Alport syndrome. Am J Hum Genet 59:1221–1232
47. Churg J, Sherman RL (1973) Pathologic characteristics of hereditary nephritis. Arch Pathol 95:374–379
48. Hinglais N, Grunfeld J-P, Bois LE (1972) Characteristic ultrastructural lesion of the glomerular basement membrane in progressive hereditary nephritis (Alport's syndrome). Lab Invest 27:473–487
49. Spear GS, Slusser RJ (1972) Alport's syndrome: emphasizing electron microscopic studies of the glomerulus. Am J Pathol 69: 213–222
50. Jansen B, Thorner P, Baumal R, Valli V, Maxie MG, Singh A (1986) Samoyed hereditary glomerulopathy (SHG): evolution of splitting of glomerular capillary basement membranes. Am J Pathol 125:536–545
51. Rumpelt H-J (1980) Hereditary nephropathy (Alport syndrome): correlation of clinical data with glomerular basement membrane alterations. Clin Nephrol 13:203–207
52. Meleg-Smith S, Magliato S, Cheles M, Garola RE, Kashtan CE (1998) X-linked Alport syndrome in females. Hum Pathol 29:404–408
53. Plant KE, Green PM, Vetrie D, Flinter FA (1999) Detection of mutations in *COL4A5* in patients with Alport syndrome. Hum Mutat 13:124–132
54. Nakanishi K, Iijima K, Kuroda N, Inoue Y, Sado Y, Nakamura H, Yoshikawa N (1998) Comparison of α5(IV) collagen chain expression in skin with disease severity in women with X-linked Alport syndrome. J Am Soc Nephrol 9:1433–1440
55. Lees G, Helman RG, Kashtan CE, Michael AF, Homco LD, Millichamp NJ, Camacho ZT, Templeton JW, Ninomiya Y, Sado Y, Kim Y (1999) A new form of X-linked dominant hereditary nephritis in dogs. Am J Vet Res 60:373–383
56. Guo C, Van Damme B, Vanrenterghem Y, Devriendt K, Cassiman J-J, Marynen P (1995) 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 95:1832–1837
57. Vetrie D, Flinter F, Bobrow M, Harris A (1992) X inactivation patterns in females with Alport's syndrome: a means of selecting against a deleterious gene? J Med Genet 29:663–666
58. Kapoor S, Dasgupta J (1979) Chromosomal anomaly in a female patient with anterior lenticonus. Ophthalmologica 179: 271–275
59. Gregory MC, Atkin CL (1993) Alport syndrome. In: Schrier RW, Gottschalk CW (eds) Diseases of the kidney. Little Brown, Boston, pp 571–591
60. Renieri A, Meroni M, Sessa A, Battini G, Serbelloni P, Torri-Tarelli L, Seri M, Galli L, De Marchi M (1994) Variability of clinical phenotype in a large Alport family with gly1143-ser change of collagen α5(IV) chain. Nephron 67:444–449
61. Grunfeld J-P, Noel LH, Hafez S, Droz D (1985) Renal prognosis in women with hereditary nephritis. Clin Nephrol 23: 267–271
62. Barker DF, Pruchno CJ, Xiang X, Atkin CL, Stone EM, Deison JC, Fain PR, Gregory MC (1996) A mutation causing Alport syndrome with tardive hearing loss is common in the western United States. Am J Hum Genet 58:1157–1165

63. Finielz P, Cartault F, Chuet C, Genin R (1998) Alport syndrome in Reunion Island: phenotypic heterogeneity of the recessive-autosomal form. Nephron 79:237
64. Pochet JM, Bobrie G, Landais P, Goldfarb B, Grunfeld J-P (1989) Renal prognosis in Alport's and related syndromes: influence of the mode of inheritance. Nephrol Dial Transplant 4:1016–1021
65. Harvey SJ, Zheng K, Sado Y, Naito I, Ninomiya Y, Jacobs RM, Hudson BG, Thorner PS (1998) Role of distinct type IV collagen networks in glomerular development and function. Kidney Int 54:1857–1866
66. Cosgrove D, Samuelson G, Meehan DT, Miller C, McGee J, Walsh EJ, Siegel M (1998) Ultrastructural, physiological, and molecular defects in the inner ear of a gene knockout mouse model of autosomal Alport syndrome. Hear Res 121:84–98
67. Muda AO, Rahimi S, Renieri A, Rizzoni G, Massella L, Faraggiana T (1997) Ultrastructural immunocytochemistry of collagenous and non-collagenous proteins in fast-frozen, freeze-substituted, and low-temperature-embedded renal tissue in Alport syndrome. J Pathol 182:465–474
68. Kashtan CE, Gubler MC, Sisson-Ross S, Mauer M (1998) Chronology of renal scarring in males with Alport syndrome. Pediatr Nephrol 12:269–274
69. Callis L, Vila A, Carrera M, Nieto J (1999) Long-term effects of cyclosporine A in Alport's syndrome. Kidney Int 55: 1051–1056
70. Kashtan CE, McEnery PT, Tejani A, Stablein DM (1995) Renal allograft survival according to primary diagnosis: a report of the North American Pediatric Renal Transplant Cooperative Study. Pediatr Nephrol 9:679–684
71. Peten E, Pirson Y, Cosyns J-P, Squifflet J-P, Alexandre GPJ, Noel L-H, Grunfeld J-P, Ypersele de Strihou C van (1991) Outcome of thirty patients with Alport's syndrome after renal transplantation. Transplantation 52:823–826
72. Gobel J, Olbricht CJ, Offner G, Helmchen U, Repp H, Koch KM, Frei U (1992) Kidney transplantation in Alport's syndrome: long-term outcome and allograft anti-GBM nephritis. Clin Nephrol 38:299–304
73. Noel LH, Gubler MC, Bobrie G, Savage COS, Lockwood CM, Grunfeld JP (1989) Inherited defects of renal basement membranes. Adv Nephrol 18:77–94
74. Hayes DK, Majeski JA, Alexander JW, Rajagopalan PR, Fitts CT, First MR, Munda R, Fidler JP (1985) Renal transplantation in Alport's syndrome. Am Surg 51:414–417
75. Berardinelli L, Pozzoli E, Raiteri M, Canal R, Tonello G, Tarantino A, Vegeto A (1990) Renal transplantation in Alport's syndrome: personal experience in twelve patients. Contrib Nephrol 80:131–134
76. McCoy RC, Johnson HK, Stone WJ, Wilson CB (1982) Absence of nephritogenic GBM antigen(s) in some patients with hereditary nephritis. Kidney Int 21:642–652
77. Brainwood D, Kashtan C, Gubler MC, Turner AN (1998) Targets of alloantibodies in Alport anti-glomerular basement membrane disease after renal transplantation. Kidney Int 53: 762–766
78. Kalluri R, Weber M, Netzer K-O, Sun MJ, Neilson EG, Hudson BG (1994) *COL4A5* gene deletion and production of post-transplant anti-α3(IV) collagen alloantibodies in Alport syndrome. Kidney Int 45:721–726
79. Dehan P, Van Den Heuvel LPWJ, Smeets HJM, Tryggvason K, Foidart J-M (1996) Identification of post-transplant anti-a5(IV) collagen alloantibodies in X-linked Alport syndrome. Nephrol Dial Transplant 11:1983–1988
80. Heuvel LPWJ van de, Schroder CH, Savage COS, Menzel D, Assmann KJM, Monnens LAH, Veerkamp JH (1989) The development of anti-glomerular basement membrane nephritis in two children with Alport's syndrome after renal transplantation: characterization of the antibody target. Pediatr Nephrol 3: 406–413
81. Ding J, Zhou J, Tryggvason K, Kashtan CE (1994) *COL4A5* deletions in three patients with Alport syndrome and post-transplant antiglomerular basement membrane nephritis. J Am Soc Nephrol 5:161–168
82. Antignac C, Knebelmann B, Druout L, Gros F, Deschenes G, Hors-Cayla M-C, Zhou J, Tryggvason K, Grunfeld J-P, Broyer M, Gubler M-C (1994) 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 93:1195–1207
83. Torra R, Badenas C, Cofan F, Callis L, Perez-Oller L, Darnell A (1999) Autosomal recessive Alport syndrome: linkage analysis and clinical features in two families. Nephrol Dial Transplant 14:627–630
84. Antignac C, Heidet L (1996) Mutations in Alport syndrome associated with diffuse esophageal leiomyomatosis. Contrib Nephrol 117:172–182
85. Antignac C, Zhou J, Sanak M, Cochat P, Roussel B, Deschenes G, Gros F, Knebelmann B, Hors-Cayla M-C, Tryggvason K, Gubler M-C (1992) Alport syndrome and diffuse leiomyomatosis: deletions in the 5' end of the *COL4A5* gene. Kidney Int 42:1178–1183
86. Zhou J, Mochizuki T, Smeets H, Antignac C, Laurila P, Paepe A de, Tryggvason K, Reeders ST (1993) Deletion of the paired α5(IV) and α6(IV) collagen genes in inherited smooth muscle tumors. Science 261:1167–1169
87. Heidet L, Dahan K, Zhou J, Xu Z, Cochat P, Gould JDM, Leppig KA, Proesmans W, Guyot C, Guillot M, Roussel B, Tryggvason K, Grunfeld JP, Gubler MC, Antignac C (1995) Deletions of both α5(IV) and α6(IV) collagen genes in Alport syndrome and in Alport syndrome associated with smooth muscle tumors. Hum Mol Genet 4:99–108
88. Heidet L, Cohen-Solal L, Boye E, Thorner P, Kemper MJ, David A, Larget Piet L, Zhou J, Flinter F, Zhang X, Gubler MC, Antignac C (1997) Novel *COL4A5/COL4A6* deletions and further characterization of the diffuse leiomyomatosis-Alport syndrome (DL-AS) locus define the DL critical region. Cytogenet Cell Genet 78:240–246
89. Segal Y, Peissel B, Renieri A, Marchi M de, Ballabio A, Pei Y, Zhou J (1999) LINE-1 elements at the sites of molecular rearrangements in Alport syndrome-diffuse leiomyomatosis. Am J Hum Genet 64:62–29
90. Ueki Y, Naito I, Oohashi T, Sugimoto M, Seki T, Yoshioka H, Sako Y, Sato H, Sawai T, Sasaki F, Matsuoka M, Fukuda S, Ninomiya Y (1998) Topoisomerase I and II consensus sequences in a 17-kb deletion junction of the *COL4A5* and *COL4A* genes and immunohistochemical analysis of esophageal leiomyomatosis associated with Alport syndrome. Am J Hum Genet 62:253–261
91. Heiskari N, Zhang X, Zhou J, Leinonen A, Barker D, Gregory M, Atkin CL, Netzer KO, Weber M, Reeders S, Grohagenriska C, Neumann HPH, Trembath R, Tryggvason K (1996) Identification of 17 mutations in ten exons in the *COL4A5* collagen gene, but no mutations found in four exons in *COL4A6*: a study of 250 patients with hematuria and suspected of having Alport syndrome. J Am Soc Nephrol 7:702–709
92. Wester DC, Atkin CL, Gregory MC (1995) Alport syndrome: clinical update. J Am Acad Audiol 6:73–79
93. Colville D, Savige J, Morfis M, Ellis J, Kerr P, Agar J, Fasset R (1997) Ocular manifestations of autosomal recessive Alport syndrome. Ophthalmic Genet 18:119–128
94. Colville DJ, Savige J (1997) Alport syndrome: a review of the ocular manifestations. Ophthalmic Genet 18:161–173
95. Nielsen CE (1978) Lenticonus anterior and Alport's syndrome. Arch Ophthalmol 56:518–530
96. Brownell RD, Wolter JR (1964) Anterior lenticonus in familial hemorrhagic nephritis: demonstration of lens pathology. Arch Ophthalmol 71:481–483
97. Kato T, Watanabe Y, Nakayasu K, Kanai A, Yajima Y (1998) The ultrastructure of the lens capsule abnormalities in Alport's syndrome. Jpn J Ophthalmol 42:401–405
98. Streeten BW, Robinson MR, Wallace R, Jones DB (1987) Lens capsule abnormalities in Alport's syndrome. Arch Ophthalmol 105:1693–1697
99. Perrin D, Jungers P, Grunfeld JP, Delons S, Noel LH, Zenatti C (1980) Perimacular changes in Alport's syndrome. Clin Nephrol 13:163–167

100. Polack BCP, Hogewind BL (1977) Macular lesions in Alport's disease. Am J Ophthalmol 84:533–535
101. Zylbermann R, Silverstone B-Z, Brandes E, Drukker A (1980) Retinal lesion in Alport's syndrome. J Pediatr Ophthalmol 17:255–260
102. Pajari H, Setala K, Heiskari N, Kaarianen H, Rosenlof K, Koskimies O (1999) Ocular findings in 34 patients with Alport syndrome: correlation of the findings to mutations in *COL4A5* gene. Acta Ophthalmol Scand 77:214–217
103. Sabates R, Krachmer JH, Weingeist TA (1983) Ocular findings in Alport's syndrome. Ophthalmologica 186:204–210
104. Teekhasaenee C, Nimmanit S, Wutthiphan S, Vareesangthip K, Laohapand T, Malasitr P, Ritch R (1991) Posterior polymorphous dystrophy and Alport syndrome. Ophthalmology 98: 1207–1215
105. Thompson SM, Deady JP, Willshaw HE, White RHR (1987) Ocular signs in Alport's syndrome. Eye 1:146–153
106. Rhys C, Snyers B, Pirson Y (1997) Recurrent corneal erosion associated with Alport's syndrome. Kidney Int 52:208–211
107. Burke JP, Clearkin LG, Talbot JF (1991) Recurrent corneal epithelial erosions in Alport's syndrome. Acta Ophthalmol 69: 555–557
108. Jonsson A, Renieri A, Gallagher PG, Kashtan CE, Cherniske EM, Bruttinin M, Piccini M, Vitelli F, Ballabio A, Pober B (1998) Alport syndrome, mental retardation, midface hypoplasia, and elliptocytosis: a new X linked contiguous gene deletion syndrome? J Med Genet 35:273–278
109. Piccini M, Vitelli F, Bruttini M, Pober B, Jonsson JJ, Villanova M, Zollo M, Borsani G, Ballabio A, Renieri A (1998) *FACL4*, a new gene encoding long-chain acyl-CoA synthetase 4, is deleted in a family with Alport syndrome, elliptocytosis, and mental retardation. Genomics 47:350–358
110. Vitelli F, Piccini M, Caroli F, Franco B, Malandrini A, Pober B, Jonsson J, Sorrentino V, Renieri A (1999) Identification and characterization of a highly conserved protein absent in the Alport syndrome (A), mental retardation (M), midface hypoplasia (M), and elliptocytosis (E) contiguous gene deletion syndrome. Genomics 55:335–340
111. Robson WLM, Lowry RB, Leung AKC (1994) X-linked recessive nephritis with mental retardation, sensorineural hearing loss, and macrocephaly. Clin Genet 45:314–317
112. Berheim J, Dechavanne M, Bryon PA, Lagarde M, Colon S, Pozet N, Traeger J (1976) Thrombocytopenia, macrothrombocytopathia, nephritis and deafness. Am J Med 61:145–150
113. Clare NM, Montiel MM, Lifschitz MD, Bannayan GA (1979) Alport's syndrome associated with macrothrombocytopenic thrombocytopenia. Am J Clin Pathol 72:111–117
114. Eckstein JD, Filip DJ, Watts JC (1975) Hereditary thrombocytopenia, deafness, and renal disease. Ann Intern Med 82: 639–645
115. Epstein CJ, Sahud MA, Piel CF, Goodman JR, Bernfield MR, Kushner JH, Ablin AR (1972) Hereditary macrothrombocytopenia, nephritis and deafness. Am J Med 52:299–310
116. Gutenberger J, Trygstan CW, Stiehm ER, Opitz JM, Thatcher LG, Bloodworth JMB (1970) Familial thrombocytopenia, elevated serum IgA levels and renal disease: a report of a kindred. Am J Med 49:729–741
117. Hansen MS, Behnke O, Pedersen NT, Videbaek A (1978) Macrothrombocytopenia associated with glomerulonephritis, deafness and aortic cystic media necrosis. Scand J Hematol 21: 197–205
118. Parsa KP, Lee DBN, Zamboni L, Glassock RJ (1976) Hereditary nephritis, deafness and abnormal thrombopoiesis: study of a new kindred. Am J Med 60:665–671
119. Peterson LC, Rao KV, Crosson JT, White JG (1985) Fechtner syndrome: a variant of Alport's syndrome with leukocyte inclusions and macrothrombocytopathia. Blood 65: 397–406
120. Naito I, Nomura S, Inoue S, Kagawa M, Kawai S, Gunshin Y, Joh K, Tsukidate C, Sado Y, Osawa G (1997) Normal distribution of collagen IV in renal basement membranes in Epstein's syndrome. J Clin Pathol 50:919–922

LITERATURE ABSTRACT

F. Scolari · A. Amoroso · S. Savoldi · G. Mazzola · E. Prati B. Valzorio · B.F. Viola · B. Nicola · E. Movilli · M. Sandrini M. Campanini · R. Maiorca

Familial clustering of IgA nephropathy: further evidence in an Italian population

Am J Kidney Dis (1999) 33:857-865

Several lines of evidence suggest that genetic factors have an important role in the pathogenesis of immunoglobulin A (IgA) nephropathy. We report the prevalence of familial IgA nephropathy in a referral center in northern Italy and present the data on HLA genotypes in the families identified. Twenty-six of 185 patients (14%) with IgA nephropathy investigated in Brescia, Italy, were related to at least one other patient with the disease. Restriction fragment length polymorphism (RFLP) analysis of HLA-DR beta and HLA-DQ alpha and beta genes, as well as polymerase chain reaction-based oligonucleotide typing, was performed in family members. The 26 patients with IgA nephropathy belonged to 10 families. Familial relationships between the patients varied greatly, ranging from parent-child to sib-pair to more distant familial relationships. No common nephrotoxic factor was identified in the families. The intervals separating the apparent onset of disease in relatives with IgA nephropathy varied from 8 months to 13 years. In patients with a family history of IgA nephropathy, there was an increased incidence of HLA-DRB1*08 compared with those with sporadic IgA nephropathy. The study shows that a significant number of the patients with IgA nephropathy followed up in Brescia had a family history of disease. The fact that the Italian population, an ethnic group not previously examined, also presents an increased familial susceptibility to IgA nephropathy suggests that familial predisposition is a very common finding for IgA nephropathy. Thus, clinicians should become aware that IgA nephropathy may aggregate within families in a substantial number of cases. In addition, this subgroup of patients with IgA nephropathy offers an ideal opportunity to elucidate the molecular genetics of this disease.