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SPECTRUM OF COLLAGEN TYPE IV NEPHROPATHIES: FROM THIN BASEMENT MEMBRANE NEPHROPATHY TO ALPORT SYNDROME

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SUMMARY

Alport syndrome and thin basement membrane nephropathy are common causes of persistent familial haematuria. They are associated with various mutations in type IV collagen genes. Mutations in genes, coding for α5 chain of collagen IV, cause X-linked Alport syndrome, whereas mutations in genes for α3 and α4 chains can cause the autosomal recessive and autosomal dominant type of Alport syndrome or benign familial haematuria with thin basement membrane nephropathy. In view of the wide spectrum of phenotypes, an exact diagnosis is sometimes difficult to achieve. Few studies of genotype-phenotype correlations in Alport syndrome have shown that various types of mutations may be a significant predictor of the severity of disease. Histopathologic findings in Alport syndrome vary from normal kidney to nonspecific focal segmental and global glomerular sclerosis with characteristic ultrastructural finding of thickening and splitting of the glomerular basement membrane. Thin basement membrane nephropathy is characterized by diffuse thinning of the glomerular basement membrane on an ultrastructural level, while by light microscopy glomeruli are mostly unremarkable. Because of present limitations of mutation screening techniques, kidney biopsy with mandatory ultrastructural analysis and immunohistochemistry examination for type IV collagen α chains remains a standard approach for establishing diagnosis and determining the mode of transmission of the disease.

Key words: Alport syndrome; thin basement membrane nephropathy; type IV collagen; mutations; pathology

INTRODUCTION

Alport syndrome (AS) is a hereditary disease caused by genetic defects in type IV collagen, the major component of basement membranes. Its prevalence is estimated at approximately 1:5000. A diagnosis of AS can be made if at least three of the following criteria are positive: family history of haematuria with or without progression to end-stage renal disease, progressive sensorineural deafness, characteristic ocular abnormalities (anterior lenticonus and/or maculopathy) and ultrastructural changes of the glomerular basement membrane expressed as characteristic thickening and splitting with "basket-weave pattern" [1]. The disease is genetically heterogeneous. Thin basement membrane nephropathy (TBMN) is another inherited disorder of type IV collagen and is the most common diagnosis in patients with persistent benign familial haematuria of glomerular origin. It probably affects at least 1% of the population. The condition is characterized by prominent diffuse thinning of the glomerular basement membrane on an ultrastructural level, lifelong glomerular haematuria, which may be accompanied by mild proteinuria, normal renal function and an autosomal dominant inheritance pattern [2, 3].

TYPE IV COLLAGEN

The type IV collagen protein family comprises six isotypes, the α1(IV) – α6(IV) chains, encoded by genes *COL4A1 – COL4A6*. The genes for α1 and α2 chains are located on chromosome 13, genes for α3 and α4 chains on chromosome 2, and genes for α5 and α6 chains on the X chromosome. Several studies indicate the existence of three protomers of type IV collagen in human basement membranes [2]. The α1-α1-α2(IV) protomer is found in all basement membranes. In glomerular basement membrane, the predominating protomer is α3-α4-α5(IV), which also occurs in Bowman's capsule and distal and collecting tubule basement membrane, as well as in basement membranes in the lung, eye and cochlea. The α5-α5-α6(IV) protomer is present in skin epidermal basement membrane and in kidney in Bowman's capsule and distal and collecting tubule basement membrane, but is not present in glomerular basement membrane (Table 1).

TABLE 1. Distribution of type IV collagen protomers in kidney and skin.

Protomer	Glomerular BM	Bowman's capsule	Tubules	Epidermal BM
α1-α1-α2	+	+	+ (all)	+
α3-α4-α5	+	-	+ (distal)	-
α5-α5-α6	-	+	+ (distal)	+

BM – basement membrane

GENETIC ASPECTS OF ALPORT SYNDROME AND THIN BASEMENT MEMBRANE NEPHROPATHY

There are three genetic forms of AS [2-4] (Table 2). The most common form (85%) is dominant, X-linked AS, caused by mutations in the *COL4A5* gene (Xq22) encoding for the α5 chain of type IV collagen. To date, more than 400 mutations, appearing randomly along the gene, have been identified in *COL4A5*. The pathogenic mutations are mostly private and only a few mutations have been found in more than one family. The autosomal recessive forms of AS (10-15%) are caused by mutations in both alleles of the *COL4A3* gene, encoding for the α3(IV) chain, or

TABLE 2. Genetic forms of Alport syndrome.

Alport syndrome	X-linked	Autosomal recessive	Autosomal dominant
Locus	*COL4A5*	*COL4A3* *COL4A4*	*COL4A3* *COL4A4*
Frequency	85%	10-15%	<5%
Gender effect	Yes	No	No
ESRD (50% of pts)	At age of 25 years	At age of 25 years (?)	At age of 50 years
Hearing loss	80-90%	100%	?
Ocular abnormalities	30-40%	30-40%	?

ESRD – end-stage renal disease

the *COL4A4* gene, encoding for the α4(IV) chain. A few reported patients with heterozygous mutations in *COL4A3* or *COL4A4* have exhibited a progressive nephropathy characteristic of AS. These patients have autosomal dominant AS [5]. Sporadic cases of AS may also occur.

Heterozygous mutations in *COL4A3* and *COL4A4* genes have been demonstrated in 25-40% of patients with benign familial haematuria and TBMN. It has been suggested that TBMN represents a carrier state of autosomal recessive AS. In some cases, mutations found in TBMN families are identical to those causing autosomal recessive AS when present in the homozygous or compound heterozygous form [6]. To date, more than 30 mutations in *COL4A3* and *COL4A4* genes have been described in autosomal forms of AS and more than 20 in TBMN [7].

Mutations in any of the α(IV) chains, resulting in chain defect (absence or abnormal structure of the mutated a chain), impair protomer assembly and the formation of the normal type IV collagen network. Consequently, the glomerular basement membrane is initially uniformly thin and susceptible to digestion by proteolytic enzymes [3]. Although the pathogenesis of glomerular basement membrane changes in AS has not been yet clarified, it has been hypothesized that in the later course of the disease, proteolysis and attempted reconstruction with α1-α1-α2(IV) protomer produce a thickened and multilayered glomerular basement membrane.

RENAL PATHOLOGY IN ALPORT SYNDROME AND THIN BASEMENT MEMBRANE NEPHROPATHY

By light microscopy glomeruli in TBMN and in early stages of AS appear normal or show minimal mesangial changes. At later stages of AS, widening of the mesangium with focal and segmental thickening of the capillary walls, hyaline deposits and collapse of the capillary loops become apparent. Segmental and global glomerulosclerosis accompanied by interstitial fibrosis are typical of later stages. In many cases, there are prominent interstitial foam cells, which are not specific and merely indicative of proteinuria [8].

Electron microscopy examination of kidney biopsy samples in AS demonstrates characteristic abnormalities

TABLE 3. Immunohistochemistry staining for type IV collagen a chains in normal and in Alport syndrome patient's kidney and skin.

Alport syndrome		α1(IV)	α3(IV)	α5(IV)
Normal	Glomerular BM	+	+	+
	Bowman's capsule	+	±	+
	Tubules (distal)	+	+	+
	Epidermal BM	+	-	+
X-linked	Glomerular BM	+	-/mosaic	-/mosaic
	Bowman's capsule	+	-/mosaic	-/mosaic
	Tubules (distal)	+	-/mosaic	-/mosaic
	Epidermal BM	+	-	-/mosaic
Autosomal recessive	Glomerular BM	+	-	-
	Bowman's capsule	+	-	+
	Tubules (distal)	+	-	+
	Epidermal BM	+	-	+

BM – basement membrane

of the glomerular basement membrane, expressed as irregular thinning, as well as a diagnostic, more or less widespread thickening of the glomerular basement membrane, with splitting and fragmentation of the lamina densa into several strands forming a "basket-weave" pattern. Small, electron-dense round granules are often seen within the lamellated glomerular basement membrane [2, 3, 8]. The extent of glomerular basement membrane thickening and lamellation is generally gender-dependant (in X-linked AS) and age-dependant. However, the earliest ultrastructural abnormality in patients with AS is diffuse attenuation of the glomerular basement membrane which is also typically demonstrated in patients with TBMN, irrespective of their age. It may therefore be difficult to differentiate AS and TBMN in children.

Immunohistochemical staining for type IV collagen α chains in kidney and skin biopsies is a useful diagnostic tool in patients with familial haematuria [9] (Table 3). Abnormal expression of α3(IV), α4(IV) and α5(IV) may be noted in about 80% of males and 60% of females with X-linked AS, as well as in many patients with autosomal recessive AS. The patterns determined by immunohistochemistry may even distinguish between X-linked and autosomal AS [4, 9-11]. In X-linked AS, male patients characteristically demonstrate negative staining for the α3(IV) and α5(IV) chains in the kidney and skin, while women frequently show segmental (mosaic) positive staining. However, variably positive reactions for α3(IV) and α5(IV) chains have been reported in female and, occasionally, male patients. Barsotti et al [12] showed in their study that the absence of the α3(IV) chain in the glomerular basement membrane could indicate a more severe renal disease in AS. In autosomal recessive AS, males and females typically show negative staining for the α3(IV) chain, while the α5(IV) chain is negative in the glomerular basement membrane and positive in the distal tubules and Bowman's capsule, as well as in the epidermal basement membrane, due to the presence of normal α5-α5-α6 trimer at these locations. It should be pointed out that normal staining for type IV collagen α chains does not exclude AS. Immunohistochemistry for a(IV) chains is normal in

TBMN. Standard immunoflurescence for assessment of immune reactant deposits is negative or may show non-specific staining for IgM and C3 in AS.

Our experience is based on the study including 112 kidney biopsies of 102 patients of 73 families with familial haematuria [13-16]. Based on clinical data, histopathology (including electron microscopy in most biopsies and immunohistochemistry in about one third) and in 57 families including also molecular genetic analysis, a diagnosis of AS was established in 50 families, TBMN in 18 families, while in 5 families the differential diagnosis was AS or TBMN. Patients with AS were younger at the time of renal biopsy (36 male, age range 3-43 years, mean 15.9; 41 female, age range 3-53 years, mean 21.3) than those with TBMN (12 male, age range 7-41, mean 20.8; 8 female, age range 8-42 years, mean 22.1). In biopsies of AS, glomerulosclerosis and characteristic ultrastructural thickening and splitting of the glomerular basement membrane were more frequently found in male patients than in female (58% vs. 46% and 32% vs. 12%), while exclusively thin basement membrane predominated in female patients (11% vs. 2%).

GENOTYPE-PHENOTYPE CORRELATION

Gross et al [17] proposed that the different effects of various mutations may be a significant predictor of the severity of disease. They classified male patients with X-linked AS into three cohorts:

- large rearrangements, frame shift, nonsense, and splice donor mutations were associated with a severe type of AS with end stage renal disease at ~20 years, 80% hearing loss and 40% ocular lesions;
- non-glycine XY-missense, glycine-XY involving exons 21-47, in-frame deletions/insertions and acceptor splice site mutations caused a moderate-severe type of AS with end stage renal disease at ~26 years, 65% hearing loss, 30% ocular lesions;
- glycine-XY mutations involving exons 1-20 were associated with a moderate type of AS with end stage renal disease at ~30 years, 70% hearing loss and 30% ocular lesions.

Similarly, Jais et al [18], in their study including 329 families, reported genotype-phenotype correlations with regard to major rearrangements and "small mutations" in male patients with AS. Furthermore, the authors also correlated genotype-phenotype in female patients and suggested a large intrafamiliar heterogeneity in girls and women, presumably due to the influence of random X-chromosomal inactivation [19]. In their opinion, prediction of the renal course in female patients with AS from genetic studies is thus impossible. It is of interest that few studies have provided evidence of organ selective X chromosome inactivation in female patients with AS, which may explain the unexpectedly progressive course of renal disease in some of them [20, 21].

Our results of genotype-phenotype correlations [22], based on the study of 17 families with AS and 40 families with benign familial haematuria from the Slovenian population of 2 million, were generally in accordance with the classification proposed by Gross et al. Among exceptions, the p.G624D mutation, which is located in the non-collagenous domain and according to Gross may be responsible for a moderate-severe type of AS, should be highlighted. In our study, it was found in 6 unrelated families with mild disease. This mutation has already been previously reported in two different families with AS, in studies including patients from Denmark, Germany, Iceland, Sweden and United States [23] and patients from the United States [24], respectively. In our study, this mutation was demonstrated in a 47-year-old female patient with a diagnosis of AS confirmed by electron microscopy of the kidney biopsy. Her brother had end-stage renal failure at the age of 45. The same mutation was also found in 5 families with a clinical picture of benign familial haematuria. However, the finding by electron microscopy of a thin basement membrane in a 16-year-old member of one family is not convincing confirmation of the benign nature of the disease. Conversely, isolated haematuria with normal renal function in two male patients at the age of 42 and 46 years, respectively, of two other families, seems to be a significant argument in favour of benign familial haematuria associated with *COL4A5* mutation. In the opinion of Gregory, a diagnosis of TBMD is fallible unless the family contains several examples of elderly haematuric males with normal renal function [25].

It is generally believed that mutations of the *COL4A5* gene cause AS, which may vary in the severity of clinical manifestations and progression of renal disease in relation to the type of mutation [9]. However, our finding of the missense mutation p.G624D of *COL4A5* in a family with a progressive form of AS, as well as in families affected with benign familial haematuria, suggests the possibility of significantly different phenotypes associated with the same gene mutation. It could be speculated that members of the five families are affected with a very mild form of AS, but we tend more to the hypothesis that AS and benign familial haematuria may represent two opposite poles of a spectrum of hereditary *COL4A5* nephropathies, similar to hereditary nephropathies associated with *COL4A3* and *COL4A4* heterozygous mutations.

CONCLUSION

In patients with familial haematuric syndrome diagnosis of AS and determination of the mode of transmission are important for prognosis and genetic counselling. Mutation screening would be theoretically the best approach, but there are still some important limitations of this technology. Evaluation of kidney biopsy including immunohistochemistry for the type IV collagen α chains remains a useful diagnostic tool.

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СПЕКТАР НЕФРОПАТИЈА КОЛАГЕНА ТИПА *IV*: ОД НЕФРОПАТИЈЕ ТАНКЕ БАЗАЛНЕ МЕМБРАНЕ ДО АЛПОРТОВОГ СИНДРОМА

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КРАТАК САДРЖАЈ

Алпортов синдром и нефропатија танке базалне мембране су чести узроци перзистентне фамилијарне хематурије. Удружени су с различитим мутацијама гена за колаген типа *IV*. Мутације гена који кодирају ланац α5 колагена *IV* изазивају Алпортов синдром везан за *X*-хромозом, док мутације гена за ланце α3 и α4 могу довести до аутозомно рецесивног и аутозомно доминантног Алпортов синдрома или бенигне фамилијарне хематурије с нефропатијом танке базалне мембране. С обзиром на то да је спектар фенотипова веома широк, понекад је тешко поставити тачну дијагнозу. У неколико студија корелације генотип–фенотип установљено је да различити типови мутација могу да буду значајни показатељи тежине болести. Хистопатолошки налази код Алпортовог синдрома варирају од нормалног налаза до неспецифичне фокалне сегментне и опште гломерулске склерозе, с типичним ултраструктурним налазом задебљања и расло-јавања гломеруларне базалне мембране. Нефропатију танке базалне мембране одликује дифузно истањење гломеруларне базалне мембране на ултраструктурном нивоу, док су на светлосномикроскопском прегледу гломерули углавном непромењени. Због садашњих ограничења у техникама скрининга мутација, биопсија бубрега с неопходном ултраструктурном анализом и имунохистохемијским прегледом на α ланце колагена типа *IV* остаје стандардни приступ код постављања дијагнозе и утврђивања начина наслеђивања болести.

Кључне речи: Алпортов синдром; нефропатија танке базалне мембране; колаген типа *IV*; мутације; патологија

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