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#### HUMAN MUTATION 9:477-499 (1997)

### REVIEW ARTICLE/MUTATION UPDATE

## The Clinical Spectrum of Type IV Collagen Mutations

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Clinical manifestations of type IV collagen mutations can vary from the severe, clinically and genetically heterogeneous renal disorder, Alport syndrome, to autosomal dominant familial benign hematuria. The predominant form of Alport syndrome is X-linked; more than 160 different mutations have yet been identified in the type IV collagen α5 chain (COL4A5) gene, located at Xq22–24 head to head to the COL4A6 gene. The autosomal recessive form of Alport syndrome is caused by mutations in the COL4A3 and COL4A4 genes, located at 2q35-37. Recently, the first mutation in the COL4A4 gene was identified in familial benign hematuria. This paper presents an overview of type IV collagen mutations, including eight novel COL4A5 mutations from our own group in patients with Alport syndrome. The spectrum of mutations is broad and provides insight into the clinical heterogeneity of Alport syndrome with respect to age at renal failure and accompanying features such as deafness, leiomyomatosis, and anti-GBM nephritis. Hum Mutat 9:477-499, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: Alport syndrome; type IV collagen gene mutations; hereditary nephritis

#### **STRUCTURE AND FUNCTION OF**

The six  $\alpha(IV)$  chains interact in different tissues

#### **TYPE IV COLLAGEN**

Type IV collagen is a multimeric protein composed of three so-called  $\alpha$  chains. To date, six different  $\alpha$ chains have been identified ( $\alpha 1 - \alpha 6$ ) with molecular weights of 170–185 kDa. Each  $\alpha(IV)$  chain consists of a central collagenous domain of about 1,400 residues containing Gly-X-Y repeat sequences interspersed by short interruptions. X and Y mainly represent proline or lysine residues that are extensively hydroxy- and glycosylated. The N-terminal 20-30 amino acids, the 7S-domain, contain a signal peptide. The C-terminal noncollagenous (NC) domain of about 230 residues consists of two homologous halves each containing six cysteine residues (Pihlajaniemi et al., 1985). Type IV collagen chains associate intracellularly into triple helices. Assembly starts at the NC domain by disulfide bridge formation and progresses toward the N-terminus (Fig. 1). Triple helical type IV collagen molecules are secreted to form a supramolecular network through dimerization at the NC domains and tetramerization at the 7S domains (Siebold et al., 1988; Timpl, 1989). Additional lateral chain associations provide further strength to the type IV collagen network (Yurchenco and Ruben, 1987).

in different combinations. The  $[\alpha 1]2\alpha 2$  (IV) and  $[\alpha 1]$  (IV) hetero- and homotrimers are ubiquitously present in all basement membranes (Hudson et al., 1993a). The  $\alpha 3(IV)$ ,  $\alpha 4(IV)$ ,  $\alpha 5(IV)$ , and  $\alpha 6(IV)$ chains are minor basement membrane components with restricted tissue distribution. The basement membranes of the glomerulus, inner ear, lung, eye, and seminiferous tubule consist of two separate networks, one consisting of  $\alpha 1$  and  $\alpha 2$  heterotrimers and one of a tissue-specific combination of the other chains (Kleppel et al., 1992; Hudson et al., 1993). For example, the  $\alpha 1(IV)$  and  $\alpha 2(IV)$  chains reside in the subendothelial layer of the glomerular basement membrane (GBM), while the  $\alpha 3$  (IV),  $\alpha 4$  (IV), and  $\alpha 5$  (IV) chains are particularly prominent in the lamina densa (Kleppel et al., 1989a) (Fig. 1). The high cysteine content of the  $\alpha 4(IV)$  chain, probably involved in intermolecular assembly, may result in

#### additional strength to the GBM. The $\alpha 3(IV)$ and $\alpha$ 4(IV) chains colocalize in a large set of basement

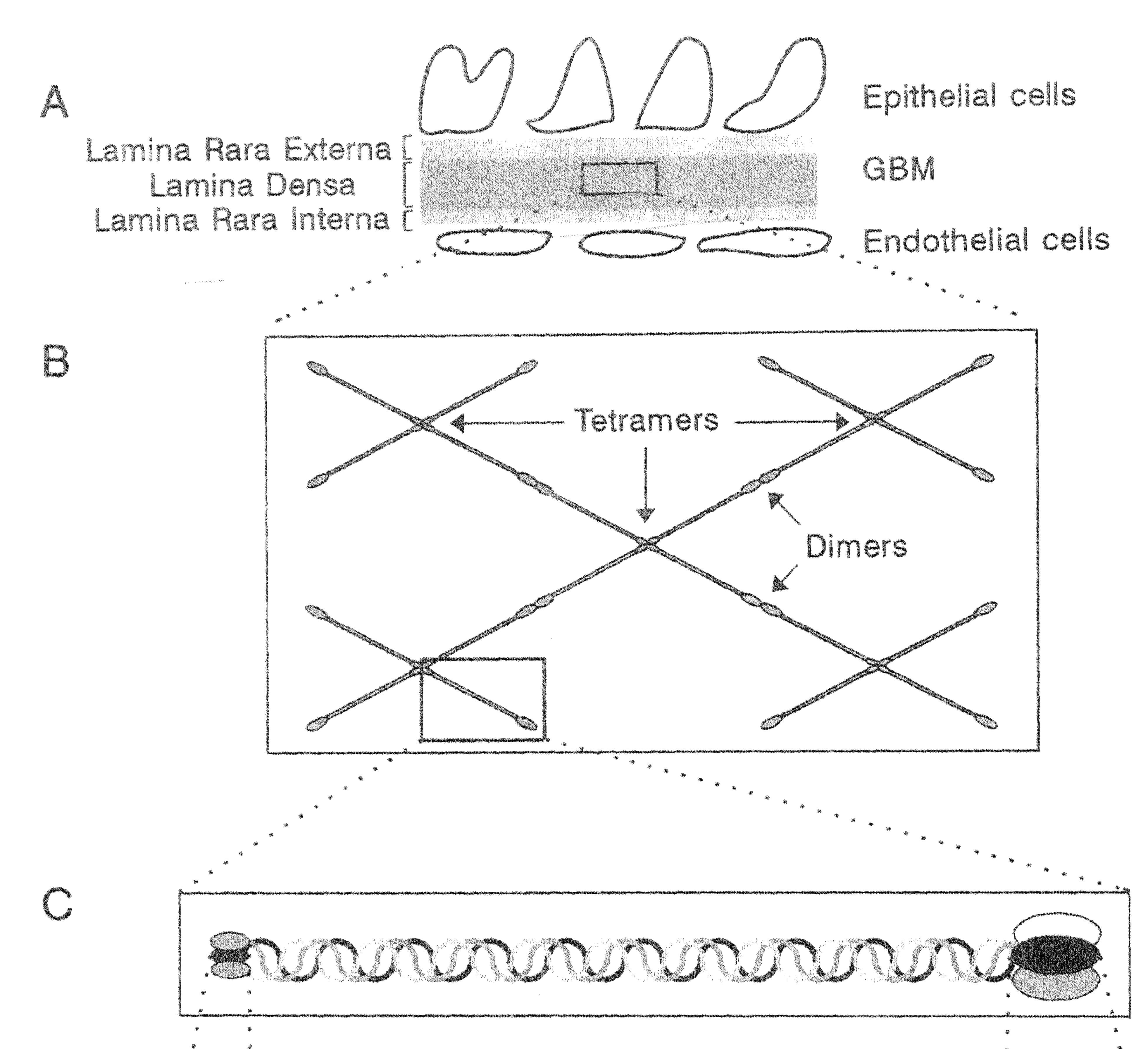
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Suprastructure of Type IV collagen

Type IV collagen protomer

# john man im

Type IV collagen chain

76 N-terminus collagenous domain

# NC domain C-terminus

FIGURE 1. Schematic representation of the molecular structure of type IV collagen in renal glomerular basement membrane. A: The glomerular basement membrane (GBM) is located between epithelial and endothelial cell layers. B:

membranes but, in contrast to the  $\alpha 5$ (IV) chain, are absent from the epidermal basement membrane (Kleppel et al., 1989a; Sanes et al., 1990; Yoshioka et al., 1994). The  $\alpha 5$ (IV) and  $\alpha 6$ (IV) chains do not always colocalize, and the  $\alpha 6$ (IV) chain is absent from Supramolecular network of type IV collagen is formed by assembly of dimers (NC-NC domain) and tetramers (7S-7S domain). C: Three  $\alpha$ (IV) chains associate into a triple helical protomer.

and C-terminal region of the  $\alpha 3$ (IV) NC1 domain contribute to the formation of the Goodpasture epitope that specifically bind the autoimmune antibodies in patients with Goodpasture syndrome (Kalluri et al., 1991; Quinones et al., 1992).

the GBM (Ninomiya et al., 1995).

Type IV collagen is the major structural component of mammalian basement membranes that serves as a scaffold for binding and alignment of other macromolecules, such as laminin, heparan sulfate proteoglycan, and nidogen (Timpl, 1989). It has a role as a physical barrier between different tissues and functions in cellular processes like filtration by the GBM. Moreover, extracellular matrix proteins such as type IV collagen mediate through membranebound receptors adhesion, differentiation, cell signaling, and tissue regeneration (Kühn, 1994). The N-

## TYPE IV COLLAGEN GENES AND EXPRESSION

The complete cDNAs for six distinct human type IV collagen chains, varying in length from 6 to 10 kb, have been characterized. The COL4A1 and COL4A5 gene comprise about 100–250 kb and contain, respectively, 52 and 51 exons (Soininen et al., 1989; Zhou et al., 1994a). The different number of exons has no functional significance as the 133-bp exon 19 in COL4A5 is a fusion product of the smaller exons 19 and 20 in COL4A1. For the other COL4 genes, only part of the intron–exon structure has been eluci-

#### CLINICAL SPECTRUM OF TYPE IV COLLAGEN MUTATIONS 479

dated (Hostikka and Tryggvason, 1987; Quinones et al., 1992; Kamagata et al., 1992; Sugimoto et al., 1993). Type IV collagens are arranged in pairs on human chromosome 13q35–37 (COL4A1/COL4A2) (Boyd et al., 1986), on 2q35-37 (COL4A3/ COL4A4) (Mariyama et al., 1992), and on Xq22-24 (COL4A5/COL4A6) (Zhou et al., 1993a). Homology studies suggest an evolution in higher organisms from an ancestor gene, which is duplicated and inverted, followed by duplication of the gene pair to other chromosomes (Zhou et al., 1994b; Leinonen et al., 1994). The  $\alpha$ 1-class consists of COL4A1, COL4A3, and COL4A5, the α2-class of COL4A2, COL4A4, and COL4A6 (Zhou et al., 1994b; Zhang et al., 1996). The two classes differ in the number of exons, encoding the NC domain, which is 5 for the  $\alpha$ 1 class and 3 for  $\alpha$ 2. The NC and 7S domains are strongly conserved through evolution, although all chains differ at amino acid positions 1611–1630 and 1721–1747 of the NC domain (Leinonen et al., 1994). Eighteen cysteine residues are conserved among all chains. The type IV collagen gene pairs share a promoter region (Zhou et al., 1994b). The COL4A1 and COL4A2 genes are expressed ubiquitously, whereas the other chains have a restricted distribution (Kleppel et al., 1989a; Sanes et al., 1990). The expression pattern of the COL4A3 and COL4A4 genes indicates coregulation (Miner and Sanes, 1994), which is not the case for the COL4A5 and COL4A6 genes (Zhou et al., 1993a). Alternative splicing has been observed for COL4A5 (Guo et al., 1993) and COL4A3, although the significance is unclear (Bernal) et al., 1993). Analysis of the 5' end of COL4A6 revealed two alternative promoters that control the generation of two different transcripts (Sugimoto et al., 1994). Experimental data on the developmental regulation of type IV collagen gene expression in the kidney are available predominantly from rat embryos. Initially, only the  $\alpha 1$  and  $\alpha 2$  (IV) chains are detected. Next, the  $\alpha 1 - \alpha 5$  (IV) chains are present and finally  $\alpha 3-\alpha 5$  (IV) become the predominant chains (Miner and Sanes, 1994). Previous studies on fetal human kidney development provided preliminary evidence for a similar switch in humans (Kleppel and Michael, 1990), although all five chains remain present in the adult GBM (Hudson et al., 1993).

juvenile (before 31 years) or adult age (older than 31 years) (Atkin et al., 1988). Clinical features are usually less severe in females (Hasstedt et al., 1986; Flinter et al., 1988). Alport syndrome is clinically heterogeneous, and patients have been classified by their age at end-stage renal disease (ESRD) and by the presence of accompanying features, such as sensorineural deafness and ocular lesions (lenticonus and perimacular flecks) (Sohar, 1956; Perrin et al., 1980). Juvenile cases are with a single exception deaf and show ocular abnormalities. More rarely, patients develop diffuse esophageal and vulvar leiomyomatosis (Cochat et al., 1988; Garcia-Torres and Orozco, 1993) or macrothrombocytopenia (Epstein et al., 1972; Peterson et al., 1985). Among patients with diffuse leiomyomatosis, severe congenital and bilateral cataracts are frequent (see Table 3). Alport patients who reach ESRD are dialyzed or undergo renal transplantation. Some transplanted patients develop a posttransplant anti-GBM nephritis, leading to irreversible graft failure (Cameron, 1991).

#### **Electron Microscopic and Immunohistochemical** Analysis of Alport Kidneys

Electron microscopic analysis of renal biopsies of Alport patients show an irregular thinning/thickening and multilamellation of the GBM. Young patients often show only thinning (Spear and Slusser, 1972; Gubler et al., 1976). The involvement of type IV collagen in Alport syndrome was indicated by immunohistochemical analysis of renal biopsies using anti-type IV collagen antibodies. The anribodies directed against type IV  $\alpha$ 3 and  $\alpha$ 5 collagen chains did not bind to the GBM in most Alport patients (Kleppel et al., 1989b; Savage et al., 1989). Further evidence came from studies of collagenase treated renal basement membranes from Alport patients, in which type IV collagen NC domains were absent (Kleppel et al., 1987; Thorner et al., 1990). The type IV collagen COL4A3 and COL4A5 NC domains were also the targets of anti-GBM antibodies, which occurred in some patients after renal transplantation (Van den Heuvel et al., 1989; Kashtan et al., 1990). These data also point to a possible type IV collagen defect as the cause of Alport syndrome.

#### **Alport Genetics**

#### **ALPORT SYNDROME**

#### **Clinical Features**

The combination of a progressive hereditary nephritis with sensorineural deafness was first described by Alport in 1927. Alport syndrome is characterized by hematuria progressing in males to renal failure at

The estimated gene frequency of Alport syndrome is 1:5,000. The disorder is genetically heterogeneous (Hasstedt et al., 1986), but the vast majority (85%) of Alport pedigrees showed X-linked dominant inheritance. The X-linked Alport gene was mapped to the Xq22-24 region (Atkin et al., 1988; Brunner et al., 1988; Flinter et al., 1989), in which the COL4A5

and COL4A6 genes were subsequently localized (Hostikka et al., 1990). Mutations in the COL4A5 gene turned out to be the main cause of Alport syndrome. The autosomal recessive (AR) form comprises about 10–15% of the pedigrees and is linked to the COL4A3 and COL4A4 locus (Chan et al., 1993). Recently, mutations were identified in the COL4A3 and COL4A4 genes in AR Alport families (Mochizuki et al., 1994; Lemmink et al., 1994b). In these families, female patients were clinically indistinguishable from affected male siblings; carriers were asymptomatic and often consanguinous. Recently, the autosomal dominant form was mapped to the COL4A3 and COL4A4 locus as well (Jefferson et al., submitted), but mutations have not yet been identified.

#### MUTATION DETECTION IN TYPE IV COLLAGEN GENES

#### **Mutation Detection Techniques**

Southern blot analysis was performed using cDNA probes to identify major gene rearrangements (Netzer et al., 1992; Renieri et al., 1992b; Smeets et al., 1992; Antignac et al., 1994), in some cases using pulsefield gel electrophoresis (Boye et al., 1991; Antignac et al., 1992). A variety of methods were used to screen for small mutations. The main approach has been SSCP analysis (Orita et al., 1989), followed by sequence analysis of fragments with a different electrophoretic mobility (Lemmink et al., 1993; Renieri et al., 1993; Boye et al., 1995). Some groups have used chemical mismatch cleavage (Boye et al., 1993), denaturing gradient gel electrophoresis (DGGE) (Zhou et al., 1992a; Netzer et al., 1993), heteroduplex analysis (Peissel et al., 1994) or direct sequence analysis without any prescreening (Nomura et al., 1993). The COL4A5 gene was screened exon by exon, because the mRNA level was too low in PBL for routine investigation of large fragments of cDNA (Guo et al., 1993; Knebelmann et al., 1992). By contrast, the COL4A3 and COL4A4 genes can be studied at the cDNA level (Mochizuki et al., 1994).

#### **Diagnostic Criteria**

Flinter et al. (1988) proposed that at least three of the following four criteria were required for the diagnosis Alport syndrome:

- 1. Family history of hematuria, in most cases progressing to renal failure (ESRD)
- 2. Irregular thickening and splitting with multilamellation of the GBM
- 3. Characteristic eye lesions (i.e., anterior lenticonus and perimacular flecks)
- 4. High-tone sensorineural deafness, which is usu-

#### **COL4A5 MUTATIONS**

#### Large COL4A5 Gene Rearrangements

Thirty-eight large- and medium-size deletions

ally progressive during childhood

This is a strict definition. In most reported cases no data on eye abnormalities were available.

#### FAMILIAL BENIGN HEMATURIA

Familial benign hematuria or thin basement membrane disease is characterized by persistent hematuria, an electron microscopically detectable thin GBM and an autosomal dominant mode of inheritance. Renal function remains normal (Gauthier et al., 1989). In children differentiation between familial benign hematuria and Alport syndrome can be difficult, because both disorders manifested by persistent hematuria and thin GBM at that age. Several groups investigated whether familial benign hematuria could be a type IV collagen disorder as well. In three Japanese families, no linkage was found between the disorder and the COL4A3 and COL4A4 locus (Yamazaki et al., 1995). By contrast, linkage was found in a large Dutch family and a pathogenic mutation was found in the COL4A4 gene (Lemmink et al., 1996). These results indicate that familial benign hematuria is a genetically heterogeneous disorder, part of which can be explained by type IV collagen defects.

have been identified (Table 1). The size of the deletions varies from single exons to the complete COL4A5 gene, spanning a region of around 250 kb of genomic DNA (Table 1). The intragenic deletions were scattered across the gene, and no frequent deletion breakpoints were characterized. Deletions spanning the 5<sup>-</sup> ends of the COL4A5 and COL4A6 genes were associated with a rare combination of Alport syndrome with diffuse esophageal and/or genital leiomyomas (Antignac et al., 1992; Zhou et al., 1993a). The deletion breakpoint is always located in intron 2 of the COL4A6 gene (Zhou et al., 1993a). Alport patients with more extended deletions farther downstream COL4A6 do not have leiomyomatosis (Heidet et al., 1995). Thus far, no mutations have been identified in the COL4A6 gene only in patients with Alport syndrome (Zhou et al., 1993a; Heiskari

et al., 1996).

#### **Small COL4A5 Mutations**

One hundred and thirty-eight small COL4A5 mutations have been identified in Alport patients. They can be divided in either amino acid substitutions (n = 62), mutations creating premature stop

Antignac et al., 1994; Heidet et al., 1994 Antignac et al., 1992; Zhou et al., 1993; Antignac et al., 1994; Heidet et al., 1995 Heidet et al., 1995 Dahan et al., 1995 1994; Antignac et al., 1992; Zhou et al., 1993a; Antignac et al., 1994; Heidet et al., 1995 Antignac et al., 1992; Zhou et al., 1993a; Antignac et al., 1994; Heidet et al., 1995 Renieri et al., 1995 Renieri et al., 1995 1992; 1994; Antignac et al., 199 Heidet et al., 1995 Heidet et al., 1995 Antignac et al., 1994 Heidet et al., 1995 Zhou et al., 1993a Netzer et al., 1992 1995 1995 Heidet et al., 1995 Heidet et al., ] Dahan et al., ] References Antignac ntation, phritis, 3 COL4A6 3 COL4A6 at, phritis phritis 2 COL4A6, taract ageal ageal ageal t ct, matosis, cCOL4A6, taract matosis, cCOL4A6, taract matosis, cCOL4A6, taract matosis, cCOL4A6 taract cCOL4A6 taract matosis, cCOL4A6 taract matosis, cCOL4A6 taract matosis, cCOL4A6 taract matosis, cCOL4A6 taract ageal sis, cCOL4A6 taract cCOL4A6 taract ageal sis, cCOL4A6 taract cCOL4A6 taract cCOL4A6 taract cCOL4A6 taract ageal sis, cCOL4A6 taract cCOL4

(continued)

				TABLE 1. Major COL4A5	5 Gene Rear	rangeme	ents in Alport Patients <sup>a</sup>	
				Phenot	type			
No.	COL4A5	Deletion	Predicted effect on COL4A5 protein	(age of renal failure)	Deafness	GBM	Family history	Remarks
<b></b> i	Exons 1-51	del 470 kb	No COL4A5 protein		<b>+</b>	Q	÷	Renal transplanta anti-GMB neph
8	Exons 1–51	del exons 1–51	No COL4A5 protein	Juvenile (21)	+	Q	De novo mutation	Renal transplant,
က	Exons 1–32	del 210 kb <sup>b</sup>	No COL4A5 protein	Q	QN	+	De novo mutation	Diffuse leiomyom del exons 1-2 (
4	Exons 1-30	del 330 kb <sup>b</sup>	No COL4A5 protein	Juvenile (16)	÷	+	÷	congenital cata del exons 1–3 (
١Ĵ	Exons 1–18	del 210 kb <sup>b</sup>	No COL4A5 protein	ND (>4)	ł	Q	De novo mutation	Bilateral cataract, del exons 1–2 ( diffuse esophag
9	Exon 1	del exons 1–4 <sup>b</sup> (del 75 kb)	No COL4A5 protein	ĝ	g	Q	+	leiomyomas Diffuse leiomyom del exons 1-2 (
►	Exon 1	del exon 1 <sup>b</sup> (del 90 kb)	No COLAA5 protein	g	Q	÷	÷	congenital cata Diffuse leiomyom del exons 1-2 C
Ø	Exon 1	Del exons 1–32 <sup>b</sup> (del 290 kb)	No COLAA5 protein	g	Q	Q	g	temale patient Diffuse leiomymai del exons 1-2 C congenital catai
<b>6</b>	Exon 1	del <10 kb <sup>b</sup>	No COL4A5 protein	Juvenile (9)	Ļ	+	(9) - + De novo mutation	temale patient Bilateral cataract, renal transplant diffuse esophag leiomyomatosis,
10	Exon 1	del 120 kb <sup>b</sup>	No COL4A5 protein	ĝ	<b>+</b>	+	÷	del exons 1-2 C Cataract, diffuse esophageal leio del exons 1-2 C
11	Exon 1	del 15-19 kb	No COL4A5 protein	Juvenile (16)	+	+	g	Diffuse esophagea leiomyomatosis, del exons 1–2 C renal transplant
12	Exon 1	del 420 kb	No COL4A5 protein	Q	÷	Q	De novo mutation	del exons 1-4 CO
13	Exon 1	del exon 1	No COL4A5 protein	Juvenile (23)	- <b>+</b> -	+	4	del exons 1-2 CO cataract
:								

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(contined)

Antignac et al., 1994 Renieri et al., 1995 Antignac et al., 1994 Ding et al., 1994 Smeets et al., 1992 Renieri et al., 1995 Flinter, 1993 Flinter, 1993 Boye et al., 1991; Vetrie et al., 1992 Renieri et al., 1995 Renieri et al., 1995 Netzer et al., 1992 Renieri et al., 1995 Renieri et al., 1995 Renieri et al., 1995 Dahan et al., 1995 Dahan et al., 1995 Dahan et al., 1995 Dahan et al., 1995 Ding et al., 1994 References omyomatosis, atient omyomatosis, s 1-2 COL4A6 myomatosis, del exons 1–2 splantation, A nephritis in ed patients AA3 protein imyomatosis, atient plantation, I nephritis plantation plantation plantation [ nephritis

				Phenot	upe			
Ž	COL4A5	Deletion	Predicted effect on COL4A5 protein	(age of renal failure)	Deafness	GBM	Family history	Remarks
1 4		Q	No COL4A5 protein	N N	- <b>†</b> -	+	÷	ā
15	Exon 1	Q	No COL4A5 protein	Q	1	+	De novo mutation	ā
16	Exon 1	QZ	No COL4A5 protein	ND (>16)	+	+	+	Diffuse le del exo
17	Exon 1	Q	No COL4A5 protein	I	Ŧ	QN	+	Diffuse leion female na
18	Exons 2–36	del exons 2-36	Truncated COL4A5	Juvenile (25)	÷	Q	4	
19	Exons 2–19	del exons 2–19	(frameshift) Truncated COL4A5	ND (>15)	+	+	Ŧ	
20 21	Exons 4/5 Exons 4–47	ďel 4/5–26 <sup>b</sup> del exons 4–47	Truncated COL4A5 No COL4A5 protein,	Juvenile Juvenile (<20)	++ ++	+ +	+ <b>2</b>	Renal
22	Exons 4–13	del exons 4–13	Truncated COL4A5	Juvenile (27)	Q	QN	÷	two related (no COL4/ in GMB)
23	Exons 14-51	del exons 14-51	Truncated COL4A5	Juvenile (<20)	+	+	+	Rena
24	Exon 17	del exon 17	Truncated COL4A5, del 18 amino acids	Juvenile (average 24)	+	<b>+</b> -	+	
25	Exons 19–22	del exons 19-22	(intrame) Truncated COL4A5	Juvenile (average 23)	+	Q	<b>+</b>	Renal transp
26 27	Exons 20–26 Exons 20–21	del exons 20–26 <sup>b</sup> del exons 20–21	Truncated COL4A5 Truncated COL4A5	ND ND Juvenile (18)	+ +	+Q	+ <mark>2</mark>	
28	Exon 22	del exons 22–28; del evons 38–51	(intrame) truncated COL4A5	Juvenile (17)	-	+	+	Renal transp anti-GBM
29 30	Exons 28–32 Exons 34–51	exons 28 exons 34	Aberrant COL4A5 Truncated COL4A5	Juvenile (23) ND	+ Đ	+ <mark>Q</mark>	+ <mark>2</mark>	
31	Exon 38	n Xa	Truncated COL4A5	Juvenile (26)	<b>-†</b> -	QN	+	
32 33	Exons 38–46 Exons 38–51	(34 kD deletion) Ins exons 38–46 dup 35 kb of 14 exons at 3 end	Truncated COL4A5 Aberrant COL4A5	ND Adult (33)	<b>£</b> +	<b>g</b> +	It (33) ND ND + + De novo mutation	Renal transp

			Phenotype	ype				
No. COL4A5	Deletion	Predicted effect on COL4A5 protein	ESRD (age of renal failure)	Deafness	GBM	Family history	Remarks	References
34 Exons 42-47	15-kb deletion	Truncated COL4A5; del 240 C-terminal residues	Juvenile	÷	Q	<b>+</b>		Barker et al., 1990; Zhou et al., 1991a; Zhou et al., 1992
35 Exons 45–51	del 12 kb at 3° end (del 450 kb)	Truncated COL4A5	Juvenile (13)	÷	<b>-+-</b>	÷	Renal transplantation	•
36 Ins/del 46-47		Aberrant COL4A5	ND (>16)	+	÷	+		
37 Exons 49-51		Truncated COL4A5	Juvenile (15)	÷	+	+		
38 Exon 40	del exon 40	Truncated COL4A5 (inframe)	ND (>13)	<b>+</b>	QN	<b>+</b>	Renal transplantation	Renieri et al., 1995

electron microscopy of GBM shows structural abnormalities

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ESRD, end-stage renal disease (age at renal failure or age at time report, in years); GBM, glomerular basement membrane; GBM+, diagnostic for Alport syndrome; del, deletion; ins, insertion; dup, duplication; ND, no data available.

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codons (n = 69), inframe deletions (n = 6), and one inframe duplication event. Amino acid substitutions were considered pathogenic when the underlying DNA mutations segregated with the disease in the respective families and when these mutations were not found in the general population. Additional evidence was derived from the evolutionary conservation of the altered amino acids and the nature of the change involved (Table 2). A total of 55 amino acid substitutions were detected in the collagenous domain, 49 involving glycine substitutions (Table 2). All mutated glycine residues are part of the conserved Gly-X-Y stretches in the collagenous domain. The remaining seven amino acid substitutions were located in the NC domain. Mutations in the coding region, which were part of the consensus splice site sequences (Krawczak et al., 1992), were scored as splice site mutations, and not as missense mutations. For example, substitution of the last nucleotide of exon 48 induces skipping of exon 48 at the RNA level (Lemmink et al., 1994a) and mutation of the last nucleotide of exon 49 generates a complex pattern of aberrant COL4A5 splice products (Nomura et al., 1993). A variety of small mutations created premature stop codons. Nonsense mutations, which changed an amino acid codon into a stop codon, were observed in eight cases, small deletions or insertions in 24 and 11 cases, respectively. Finally, 26 splice site mutations were detected, which destroyed highly maturia, a similar glycine substitution (Gly897Glu) was identified in the triple helical part of the protein (Lemmink et al., 1996).

#### NONPATHOGENIC AMINO ACID SUBSTITUTIONS IN TYPE IV COLLAGEN

Discrimination between pathogenic and nonpathogenic amino acid substitutions can be difficult. This is especially the case for type IV collagen, in which no test is available to determine the effect of mutations on the production and function of the protein. Here we report on the amino acid substitutions that do not fulfill the previously formulated criteria on pathogenicity. In the COL4A5 gene, Ala430Asp and the Lys664Asn substitutions were detected in Alport patients, but these mutations did not segregate with the disease and did not involve conserved amino acids (Lemmink and Smeets, unpublished data). In addition, in the patient with the Lys664-Asn substitution, a second pathogenic mutation (Pro1517Thr) was identified (Lemmink et al., 1993). Similarly, two nonpathogenic amino acid substitutions were identified in the COL4A3 gene (Leu1474Pro and Gln1495Arg). Both mutations did not segregate with the disease. The Leu1474Pro was found, although at low frequency, in the general population (Lemmink et al., 1994b). In contrast to the Gln1495, the Leu1474 residue is highly conserved in all six human type IV collagen proteins (Leinonen et al., 1994) and in differ-

conserved splice acceptor or splice donor consensus sequences (Krawczak et al., 1992). These mutations prohibited correct splicing and altered the reading frame (Nomura et al., 1993; Lemmink et al., 1994a).

#### **COL4A3 AND COL4A4 MUTATIONS**

The number of mutations in the COL4A3 and COL4A4 genes in autosomal recessive Alport syndrome is still low: six in the COL4A3 gene and three in the COL4A4 gene (Table 3), but few patients have been studied for only a small part of the gene. Patients were either homozygotes or compound heterozygotes for the mutations and their parents were described as asymptomatic carriers. All six COL4A3 mutations created a premature stop codon. Two were nonsense mutations and three were deletions involving 5 and 7 base pairs (bp), respectively (Table 3). A sixth mutation created a splice acceptor site in intron 5, counting from the 3' end of the COL4A3 gene (Knebelmann et al., 1995). The two COL4A4 mutations were a nonsense and a missense mutation that occurred as homozygotes in patients (Mochizuki et al., 1994). The missense mutation Gly1201Ser substituted a glycine in the collagenous domain. In a family with autosomal dominant familial benign heent species (Lemmink et al., 1994b) and the alteration was considered drastic. We want to stress that it is important to generate supportive evidence for the pathogenicity of amino acid substitutions.

#### EVALUATION OF COL4A5 MUTATIONS Mutation Score

A systematic analysis of large gene rearrangements, using the entire COL4A5 cDNA as a probe, has been performed for French (n = 88) families) (Antignac et al., 1994), Italian (n = 177families) (Renieri et al., 1995), German (n = 20families) (Netzer et al., 1992), and Japanese (n =60 families) (Saito et al., 1994) patients. The frequency of major rearrangements varied from 16% for the French patients to 1-2% for the Japanese. Most large rearrangements were deletions. A systematic search for small mutations in the COL4A5 gene has recently been completed in the Italian and Japanese population (Kawai et al., 1996; Renieri et al., 1996). The mutation score was about 40–45% for the Italian and Japanese population. By contrast, in the British population already 35% of the mutations were identified after screening 40% of the COL4A5 coding region

References	Renieri et al., 1996	kari et al., 1996	o et al., 1995	article	kari et al., 1996	et al., 1	ढ ढ	et al 1995	ari et a	x al., 19	ari et al., 1996 ari et al., 1996	rai et al., 1996 10 et al., 1996 1ai et al., 1996 1ai et al., 1996	kari et al., 1996 et al., 1995	ari et al., 1996
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Remarks	Other start codon,	Stop 101/102 codons	Renal transplantation, interruption Glu-X-V	Interruption Gly-X-Y, possible splice site	mutation Interruption Gly-X-Y, possible splice site	Interruption Gly-X-Y	Interruption Gly-X-Y Renal transplantation,	stop 8 codons down- stream mRNA; exon 12 spliced	out Interruption Gly-X-Y, possible splice site	mutation, renal transplantation Interruption Gly-X-Y	Kenal transplantation Stop 11 codons down- stream renal trans.	on splantation	Renal transplantation female patient, skewed X-inactivation, intermition Glu-X-Y	second COL4A5 mutation on same allele (see no.110) Interruption Gly-X-Y F
Family history	•	÷	÷	+	+	<b>.</b>	<b>≁</b> +	+	÷	- <b>-</b> +	₽ +	De novo mutation + +	- <b>∱ ↓</b>	÷
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(continued)

# TABLE 2. Small Mutations of the COL4A5 Gene in Alport Patients<sup>a</sup>

enotype<sup>c</sup>

Pher ESRD (age of renal failure)	Juvenile (28)	Juvenile (18) Adult (40)	Juvenile (30)	Juvenile (29)	ND Adult (34) Juvenile (21)	ND (>17)	Juvenile (15)	ND (>18) Juvenile (30) Juvenile (15)	Juvenile Juvenile Juvenile Juvenile	Juvenile (17) Juvenile (30)	Juvenile (15)
Effect on coding sequence <sup>b</sup>	<b>Met1Val</b>	Frameshift Gly51/ Phe52 Gly54Asp	Gly129Glu	Gly129Val	Gly174Arg Gly177Arg Frameshift Pro212	Acceptor splice site	Gly216Arg	Gly219Ser Donor splice site Frameshift Glu254	Frameshift Glu254 Acceptor splice site Acceptor splice site Glu287Stop	Glu287Stop Gly289Val	Gly292Val
Mutation	203A→G	353insA/358insT 363G→A	588G→A	588G→T	722G→C 731G→C 835/836delC	848-3C→A	848G→A	857G→A 889+ 1G→A 960/962del2	960/962del2 983-1del7 983-1del7 983-1del7 1061G→T	1061G→T 1068G→T	1077G→T
No. COL4A5	1 Exon 1	2 Exon 3 3 Exon 3	4 Exon 7	5 Exon 7	6 Exon 9 7 Exon 9 8 Exon 11	9 Intron 11	10 Exon 12	11       Exon 12         12       Intron 12         13       Exon 13	<ul> <li>14 Exon 13</li> <li>15 Intron 13</li> <li>16 Intron 13</li> <li>17 Exon 15</li> </ul>	18 Exon 15 19 Exon 15	20 Expn 15

ss     GBM     Family history     Remarks     References       +     +     +     Stop 49 codons     Heiskari et al., downstream, renal transplantation     Heiskari et al., Heiskari et al., stream, renal     Heiskari et al., Heiskari et al., transplantation       ND     +     +     +     Heiskari et al., transplantation       ND     ND     ND     Heiskari et al., transplantation       ND     ND     ND     Heiskari et al., transplantation       ND     ND     +     Heiskari et al., transplantation       ND     ND     +     Heiskari et al., transplantation       ND     +     Heiskari et al., transplantation     Heiskari et al., transplantation       ND     +     +     Heiskari et al., transplantation       ND     +     +     Heiskari et al., transplantation       ND     +     Heiskari et al., transplantation     Heiskari et al., transplantation       ND     +     +     Heiskari et al., transplantation     Heiskari et al., transplantation       ND     +     +     Herruption Gly.XY     Heiskari et al., transplantation <th></th> <th></th> <th></th> <th>Phenotype<sup>c</sup></th> <th>5</th> <th></th> <th></th> <th></th> <th></th>				Phenotype <sup>c</sup>	5				
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1234delt       Donor splice site       Juvenile (26)       +       ND       ND       Heiskard et al., Heiskard at al., Juvenile (19)       +       +       +       +       +       Heiskard et al., Heiskard et al., Heiskard et al., Heiskard at al., Juvenile (19)       + <td< td=""><td>Exon 17</td><td>1766-</td><td>Gly325Glu</td><td>ND (&gt;9)</td><td>1</td><td><b>∔</b>-</td><td>De novo mutation</td><td>Interruption ony-x-r</td><td>נמו., 1 הל הן</td></td<>	Exon 17	1766-	Gly325Glu	ND (>9)	1	<b>∔</b> -	De novo mutation	Interruption ony-x-r	נמו., 1 הל הן
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1428G $\rightarrow$ AGly409AspND (>16)+ND+ND+Interruption Gly-X-YRenieri et al., 11533T $\rightarrow$ GIle444SerJuvenile++++Kawai et al., 11533T $\rightarrow$ GIle444SerJuvenile-+++1533T $\rightarrow$ GIle444SerND-+++1533T $\rightarrow$ GIle444SerND-+++1533T $\rightarrow$ GIle444SerND-+++1542-2A $\rightarrow$ GAcceptor splice siteND (>23)+ND+This article1542-2A $\rightarrow$ GFrameshift Ile450Juvenile (13)+ND+This article1549delATFrameshift Ile450Juvenile (13)+ND+rejection, stop 1 codon	Exon 20	1482insA	Frameshift Pro428	Juvenile (23)	♣	Q	ł	19 VIIS	et al.,
15337-9GIle444SerJuvenile+++++15337-9GIle444SerJuvenile-+++Kawai et al., 115337-9GIle444SerND-++De novo mutationFemale patientKawai et al., 115337-9GIle444SerND-+De novo mutationFemale patientKawai et al., 115337-9GIle444SerND-+ND+This article1542-2A->GAcceptor splice siteND (>23)+ND+This article1549delATFrameshift Ile450Juvenile (13)+ND+rejection, stop 1 codon1549delATFrameshift Ile450Juvenile (13)+ND+rejection, stop 1 codon	Evan 90	498G_	Ghr409Asn	ND (>16)	+	ą	÷	tion	i et al.,
1533T-9GIle444SerJuvenile-+++1533T-9GIle444SerND-++De novo mutationFemale patientKawai et al., 11533T-9GIle444SerND-+De novo mutationFemale patientKawai et al., 11542-2A-9GAcceptor splice siteND (>23)+ND+This article1549delATFrameshift Ile450Juvenile (13)+ND+rejection, stop 1 codon1549delATFrameshift Ile450Juvenile (13)+ND+rejection, stop 1 codon	Even 20	533T_	lloddSor	Invenile	-	+-	+	Female patient	et al., 1
1533T->GIle444SerND-+De novo mutationFemale patientKawai et al., 11533T->GIle444SerND>+ND+This article1542-2A->GAcceptor splice siteND(>23)+ND+This article1549deIATFrameshift Ile450Juvenile (13)+ND+ND+1549deIATFrameshift Ile450Juvenile (13)+ND+rejection, stop 1 codondownstreamAcceptorAcceptorAcceptorAcceptorAcceptor	Euch 90	1523T	lloddSor	Juvenile	1	+-	+	1	et al., 19
1542-2A→G Acceptor splice site ND (>23) + ND + Multiple renal transplant Boye et al., 15 1549delAT Frameshift lle450 Juvenile (13) + ND + ND rejection, stop 1 codon downstream	Even 20	1523TC	lloddSor	<b>G</b> Z	I	+	De novo mutation	Female patient	et al., 199
1549delAT Frameshift lle450 Juvenile (13) + ND + Multiple renal transplant Boye et al., 19 rejection, stop 1 codon downstream	Latron 20		lice	ND (>23)	+	QN	+		i Ti
rejection, stop 1 codon downstream	Even 91	549AolAT	lle4	Juvenile (13)	+	QN	<b>-</b> †-	Multiple renal transplant	et al.,
		TH HONCLOT			•	I		rejection, stop 1 codon	•
								downstream	-

				Phen	Phenotype <sup>c</sup>	•			
No. CO	COL4A5	Mutation	Effect on coding sequence <sup>b</sup>	ESRD (age of renal failure)	Deafness	GBM	Family history	Remarks	References
52 Exon	on 21	1567del9	Inframe deletion	Q	QN	Q	De novo mutation	Shorter COL4A5	Renieri et al., 1996
53 Exon		1599G→A	Pro-Gly-Pro Glv466Glu	Q	÷	+	+	protein Interruption Glv-X-Y	Kawai et al. 1996
	on 22	1683G→A	Gly494Asp	ND (>20)	+	az	. +		i et al
		1685del36	Inframe deletion		÷	Q	QN	(4Å 1	et al.,
				(average 22)					
		1685del36	Inframe deletion	Juvenile (27)	÷		+	Shorter COL4A5 protein	Renieri et al., 1996
	Exon 23			enile	• <b>+</b> -		÷	inal tra	Zhou et al., 1992b
	Exon 23	1767/1768delA	Frameshift Thr 523	ON	Q	QZ	+	Stop 33 codons	Renieri et al., 1996
	Exon 24	1902G→C	Glv567Ala	Juvenile (16)	+	QN	ł	Intermintion Ghr.X.Y	Roniari at al 1006
	Intron 24		Donor splice site	<b>ND</b>	- QZ	a de la comparte de l	- ON		Renieri et al., 1220
—		₹	Gly609Val	Juvenile (23)	-	Q	QZ	<b>Renal transplantation;</b>	icle
								interruption Gly-X-Y	
	Exon 25		Gly638Val	ND(>29)	- <b>†-</b>	+ •	• <b>†</b> •	Interruption Gly-X-Y	Boye et al. 1995
	Exon 25 Evon 26		Glu653Ara	Lundrido (19)	1 -	+ -	+ →	Interruption Gly-X-Y	Boye et al., 1995
			5=		-	-		renal transplant rejection	Doye et al., 1770
	Exon 26	2219delG	Frameshift Arg673	Q	QN	QN	De novo mutation		Renieri et al., 1996
	<b>L0</b>	DOROC T	1.102.10	A.d14	_	-	-	_	
	CXOII 77	4	Giyuotvai	Junit	ŀ	<del>]-</del>	÷	female patient	hawai et al., 1996
	Exon 27	2348G→C	Donor splice site/	Juvenile	+	÷	÷	X-Y or	Kawai et al., 1996
			Gly716Arg					aberrant COL4A5 mRNA	•
	Exon 28	$\uparrow$	Gly740Glu	Juvenile	ON	÷	QN		et
	Exon 29	2517G→A	Gly772Asp	Juvenile	I	+-	÷	Interruption Gly-X-Y	•
	Exon 29	2588G→A	Gly796Arg		+	+	÷	Interruption Gly-X-Y	t al., ]
	tron 29	2597 +2delT	Donor splice site	Juvenile (17)	+	Q	+		Renieri et al., 1996
	<b>kon 31</b>	2756G→A	Gly852Arg	<u>B</u>	I	+	+	Gly-X-Y	Kawai et al., 1996
	Exon 31	2799G→A	Gly866Glu	Adult (>31)	+	QN	÷	Gly-X-Y	et al
	xon 31		Gly866Glu	ND (>7)	I	DN	+	Gly-X-Y	Renieri et al., 1996
	Exon 31		Gly869Arg	ND (>24)	÷	+	+		t al., 19
•••		2807G→A	Gly869Arg	Juvenile (10)	I	QN	÷	<u>S</u>	et al.,
	ŝ		Gly869Arg	ND (>13)	+	QZ	+	Gly-X-Y	Renieri et al., 1996
				ą	+	Q	+	Gly-X-Y	Boye et al, 1995
	Exon 31	Š.	Frameshift Ser897	Juvenile (12)	+	+	÷	al transplant	Boye et al., 1995
								rejection, stop 21 codons downstream	
	Exon 33	3004insT	Frameshift Gly935	QZ	<b>ND</b>	QZ	QZ		Hertz et al.,
								unstream	paration
	Exon 33	3025del3	Inframe deletion Ser942	Q	QN	Q	De novo mutation	Interruption Gly-X-Y, shorter COL4A5	Renieri et al., 1996
									(continued)

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TABLE 2. Small Mutations of the COL4A5 Gene in Alport Patients<sup>a</sup> (continued)

Phenotype <sup>c</sup>	Deafness	QN	+ + +	+ + Q	+ <del>2</del> +	++ I ++	Q	+	÷	·++·+++ €	2 2
Phen	ESRD (age of renal failure)	QN	ND (>20) Juvenile	(average 22) Juvenile (27) Juvenile (29) ND	Juvenile (16) ND Juvenile (23)	ND(>29) ND (>27) Juvenile (19)	QN	Adult	Juvenile	Juvenile ND ND Juvenile (17) ND ND (>31) ND (>31) ND (>31) ND (>31) ND (>24) ND (>13) ND (>13) ND (>13) ND (>13) ND (>13) ND (>12) ND (10) ND (10) ND (20) ND	R R
	Effect on coding sequence <sup>b</sup>	Inframe deletion	Pro-Gly-Pro Gly466Glu Gly494Asp Inframe deletion	Inframe deletion Gly521Cys Frameshift Thr 523	Gly567Ala Donor splice site Gly609Val	Gly638Val Gly638Ala Gly653Arg	Frameshift Arg673	Gly684Val	Donor splice site/	Gly772Asp Gly796Arg Gly855Arg Gly8666Glu Gly8666Glu Gly8669Arg Gly869Arg Gly869Arg Gly869Arg Gly869Arg Gly869Arg Gly869Arg Gly872Arg Frameshift Ser897	Inframe deletion Ser942
	Mutation	1567del9	1599G→A 1683G→A 1685del36	1685del36 1763G→T 1767/1768delA	1902G→C 1981 + 1G→T 2028G→T	2115G→T 2115G→C 2159G→A	2219delG	2253G→T	2348G→C	2517G→A 2588G→A 2597 +2deff 2799G→A 2799G→A 2807G→A 2807G→A 2807G→A 2816G→C 2816G→C 2825 del A	3025del3
	COL4A5	Exon 21	Exon 21 Exon 22 Exon 22	Exon 22 Exon 23 Exon 23	Exon 24 Intron 24 Exon 25	Exon 25 Exon 25 Exon 26	Exon 26	Exon 27	Exon 27	Exon 29 Exon 29 Exon 21 Exon 31 Exon 3	Exon 33
	No.	52	55 <del>5</del> 55 57	56 57 58	59 61 61	5 8 <del>2</del>	65	99	67	<b>00122242226</b>	81 8

Boye et al., 1995 Kawai et al., 1996 Lemmink et al., 1994a; Lemmink et al., 1993 Kawai et al, 1996 Lemmink et al., 1994a Renieri et al., 1994a Renieri et al., 1994b Hertz et al., in preparation Kawai et al., 1996 Renieri et al., 1996 Renieri et al., 1993 Renieri et al., 1993 Renieri et al., 1996 Kawai et al., 1996 Netzer et al., 1993 Zhou et al., 1992a Peissel et al., 1994 This article References emarks Reemarks Reemarks Reemarks Reemarks Reema transplantaton, Pestop 13 codons downstream top 6 codons downstream top 24 codons Gly-X-Y Read the transplantation (Gly-X-Y, Redons and the transplantation, Redons anti-GBM nephritis, mRNA: exon 38 spliced out regettion anti-GBM nephritis, mRNA: exon 38 spliced out transplantation, Renal transp

(continued)

			TABLE 2. S	Small Mutations of the C Phen	OL4AD Gene otvpe <sup>c</sup>	e in Alpoi	L'auents (continueu)	
No		Mutation	Effect on coding sequence <sup>b</sup>	ESRD (age of renal failure)	Deafness	GBM	Family history	Remarks
82	Exon 34	3141-3144delA	Framshift Gly982	Juvenile average		Q	+	Renal transp stop 13 cc downstrea
83	Exon 34	3166delG	Frameshift Asp989	ND (>9)	+	<b>+</b>	+	Stop 6 codo downstrea
84	Exon 36	3414C-→G	Ser1071Stop	Q	Q	Q	Q	Stop at code
85 86	Exon 37 Exon 37	3513G→T 3532-3533delA	Gly1104Val Frameshift Thr1111	<u>g</u> g	١ĝ	+ g	• <b>∳</b> - <b>∤</b> -	Interruption Stop 40 cod downstree
87	Exon 37	3539insCCTG	Frameshift Gly1113	ND (>3)	I	Ŋ	De novo mutation	Stop 24 cod downstrea
88	Exon 38	3629G-→A	Gly1143Ser	Adult	Ŧ	+	+	Interruption
89	Exon 38	3630G-→A	Gly1143Asp	Juvenile (25)	I	+	+	Interruption renal tran
6	Intron 38	3656 + 1G→C	Donor splice site	Juvenile (18)	+	≁	(18) + +	rejection Renal trans anti-GBM mRNA: ex
61	Exon 39	3709/3710delG	Frameshift Gly1170	ND(>19)	+	÷	+	out Stop 128 cc downstrea
92	Exon 39	3744del7	Frameshift Lys1181	Juvenile (19)	<b>+</b>	Q	÷	Renal trans stop 115
93 94	Exon 39 Exon 41	3746G→C 3813/3814/	Gly1182Arg Frameshift Gly1205	Juvenile Juvenile (16)	<b>+</b> +	+ Q	<b>⊷∳⊷</b>	Interruption Stop 93 coc downstrean
92	Exon 41	3819del4	Frameshift Asp1206	Juvenile (average 22)	÷	+	÷	Renal trans stop 91 c downstre
96	Exon 41	3912del52	Frameshift Pro1240	Q	l	÷	÷	Stop 41 coc downstre
90	Exon 41	3923G→T 2050ine4	Gly1241Cys Frameshift Aro1253	ND (>23) ND	I +	- <b>┼╴</b> ╺╇╸	De novo mutation +	Interruption
66	Intron 41	3993-1G→A		Juvenile (16)	+	<b>+</b>	÷	mRNA: exo out, stop 4129, an
								3993 intr shift with

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Deafness + ND +	RD RD RD	Family history +	Remarks Interruption Gly-X-Y Renal transplant rejection, stop 26 codor	References Hertz et al., in preparation Renieri et al., 1994a
+ ĝ	+ 9	<b>+</b> +	tream insplantatio 0 codons tream odons down	Guo et al, 1993 Renieri et al., 1996
Q +	a a	ĝ+	suream mRNA: exon 44 spliced out Interruption Gly-X-Y	Renieri et al., 1996 Renieri et al., 1996
<u> 2</u> 2 +	ee e	+ De novo mutation +	Interruption Gly-X-Y Female patient, stop 30 codons down- stream Additional Cys residue	Renieri et al., 1996 Massella et al., 1994 Renieri et al., 1996
ıı ĝ	g+ g	+ + +	Interruption Gly-X-Y Female patient, skewed X-inactivation, other COL4A5 mutation (see no. 19)	Renieri et al., 1996 Guo et al., 1995 Hamalainen et al., 1995
1 + +	+ <u>9</u> 9	ND + De novo mutation	Renal transplantation, stop at codon 1485 Renal transplantation, stop 36 codons	This article Hertz et al., 1995 Renieri et al., 1996
1 +	g+		mRNA; exon 47 spliced out, inframe del 71 amino acids	Tverskaya et al, 1996 Nakazato et al., 1995
Ⅰ♣ ∔	++ g		Stop 9 codons downstream	Kawai et al., 1996 Nakazato et al., 1993 Lemmink et al., 1993
I	Q	Q		Renieri et al., 1996 (continued)

TABLE 2.

# lenotype<sup>c</sup>

COL4A5       Mu         Exon 42       401         Exon 42       401         Exon 42       401         Exon 42       401         Exon 42       405         Exon 43       419         Exon 44       424         Exon 45       435         Exon 45       435	Mutation 4010G→A 4015/4016delC 4058del7 4192/4193delT 4200-2A→G	ding	ESRD (age of renal failure)
	10G→A 15/4016delC 58del7 92/4193delT 00-2A→G		
	15/4016delC 58del7 92/4193delT 00-2A→G	Gly1270Ser	QN
	58del7 92/4193delT 00-2A→G	Frameshift Pro1272	Juvenile (20)
	92/4193deIT 00-2A→G	Frameshift Asn1286	Juvenile (13)
	00-2A→G	Frameshift Phe 1331	ND(>8)
		Acceptor splice site	Juvenile (30)
	45del6/ 41G→A 88G→T	Inframe deletion Pro- Gly/Gly1348Glu	Q A
	63insTCCT	Frameshift Gly1388	<u>a</u> g
	30C→T	Arg1410Cys	Juvenile
	63GT 67CT	Gly1421Trp Arg1422Cys	(average 2b) Adult (>33) Juvenile (30)
	17dup36	Duplication 12 codons	g
	53G-→A 37del2	from Gly1439 Gly1451Ser Frameshift Gly1479	ND (>8) Juvenile (20)
	46insT	Frameshift 1449Pro	Juvenile (12)
		Ala1498Asp Donor splice site	Adult (37) Juvenile (18)
	12+1G→C 49insT	Donor splice site Frameshift Met1516	Juvenile Juvenile (18)
	51C→A	Pro1517Thr	Juvenile <sup>e</sup>
	51C→A	Pro1517Thr	(average 16) ND (>15)

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		Phen	Phenotype				
Mutation	Effect on coding sequence <sup>b</sup>	ESRD (age of renal failure)	Deafness	GBM	Family history	Remarks	References
	TALEQOCAL		ſ	+	+	<b>Renal transplantation</b>	
ן פו		in a la l	-	CIN I	-4		Zhou et al., 1993b
4889C→T	Arg15035top	7) annann 7	<b>⊢</b> –		4		This article
4889C→T	Arg1563Stop	nvenile (1	┢		- ·	Dane I transfer to the	Thom at al 1993h
J	Donor splice site	Juvenile (29)	╋	N	÷	Nenai transplantation	
1	Donor splice site	ND (>21)	╋	+	<b>-†</b> -		
יי כו	en line	Immedia	4	÷	+	Renal transplantation,	Lemmink et al., 1995;
489000-AA	ane and shire are	(average 25)	•	,		mRNA; exon 48 spliced	Lemmink et al., 1994a
						out, framesmit with stop 10 codons downstream	
		A.d14	1	4	De novo mutation	Female patient	Kawai et al., 1996
ņ,	Acceptor splice site		¦ -4				2
4893G→C	Cys15645er		⊦ •				
4989G→A	Glv1596Asp	ND(>19)	╋	IND	<b>+-</b>		$\frac{1}{1-1}$
0	Frameshift 1597Tvr	ND (>12)	+	+	-	Stop at codon 1597	Nakozato et al., 1754
		Juvenile (15)	1	+	+	mRNA; multiple transcripts	Nomura et al., 1993
	Met 1601 Ile					with premature stops	
CODE   1 Cinc 10	Donor enlire cite	Adult (>30)	÷	÷	+	mRNA; ins 9 bp exon 49,	et al.,
ATSHIDT + CAAC	Frameshift Met 1601					stop 110 nucleotides	Lemmink et al., 1994a
						downstream	
	Commerchift Chul611	Juvenile (12)	+	+	+	<b>Renal transplantation</b>	Renieri et al., 1993
Diancene/zene	LIQUICSING ONATOTIC		•			stop 3 codons down-	
						stream	
CIART C	I on 1649Ard	Adult (>31)	┾	÷	÷		al., 19
5170delC	Frameshift Asp1656		ł	+	De novo mutation	Female patient, stop 10 codons downstream	Kitagawa et al., 1995
2170 9A C	Accentor solice site	Juvenile (11)	╋	♣	+	mRNA; cryptic splice	Nakazato et al., 1995
ļ	our onde mide					site, 5178del10, stop 4 codon downstream	
5191del8/	Frameshift 1665Thr	Q	QN	Q	Q	Stop 8 codons	Boye et al., 1993
5195 del8					•		Nabazato of al 1994
5237C→T	Glu1679Stop	ND (>15)	1	÷	<b>+</b> -	rutation in mother	

shows electron microscopy of GMB GBM+,

Small Mutations of the COL4A5 Gene in Alport Patients<sup>a</sup> (continued) TABLE 2. d with respect to the location of the mutation. Mutations are designated according to Beaudet and Tsui (1993). umbering for human  $\alpha_5(W)$  chain is based on Zhou et al., 1994a. s of Alport syndrome, such as perimacular flecks; anterior lenticonus are not included because insufficient data were available. further downstream:  $4068A \rightarrow T$ , and  $4071C \rightarrow T$  (Guo et al., 1993). failure based on data from two deceased brothers. I disease (age at renal failure or age at time report, in years); GBM, glomerular basement membrane; del, deletion, ins, insertion es diagnostic for Alport syndrome; dup, duplication; ND, no data available.

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	<sup>3</sup> Patients are numbered <sup>3</sup> Amino acid residue nu <sup>3</sup> Amino acid residue nu <sup>4</sup> Additional features <sup>4</sup> Additional mutations <sup>4</sup> Additional mutations features f
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	H H H H H H H H H H H H H H H H H H H
	A A O A A S A

• •	-					
Exon 49 Exon 49 Exon 49 Exon 49 Exon 49	Exon 49	Exon 50	Exon 50 Exon 50	Intron 50	Exon 51	Exon 51
127 128 130 130	132	133	134 135	136	137	138

6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	n 49
H H H H H H H H H H H H H H H H H H H	Exon
122123255123	127

COL4A5

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			Pher	Phenotype			
	ې م	oding	ESRD				
	uon	sequence	(age of renal failure)	Deafness	GBM	Remarks	Roforoncoc
Exon 5 4441C homoz	4441C→T homozygous	Arg1481Stop	Juvenile (11)	÷	+	Renal transplantation, consanguinous parents	Mochizuki ot al 1004
Exons 4;5 4559C→G 441C→T compound	C C C C C C C C C C C C C C C	Ser1524Stop; Arg1481Stop	Q	Q	Q		t al., ]
Exon 5 4414del5 4419del5	heterozygote 4414del5/ 4419del5	Frameshift Leu1474	Juvenile (9)	+	÷		Mochizuki et al., 1994
Exon 5 4414del5 4419del5	homozygous 4414del5/ 4419del5	Frameshift Leu1474	ND(>5)	Q	+	stop 33 codons downstream, female patient	Lemmink et al., 1994b
Intron 1 G→T	heterozygous G⊸T	Frameshift Arg1643	Juvenile (14)	-+-	g	2 aberrant mRNAs: insertion nart Alu	
Exon 6 4346del7 homozyge	4346del7 homozygous	Frameshift 1449Thr	Juvenile (20)	<b>+</b>	÷	onsanguinous n; anti-GBM codons downstream,	Ding et al., 1995
COL4A4							
Collagenous 2298G domain heteroz Collagenous 3809G	2298G⊸A heterozygous 3809G⊸A	Gly897Glu Gly1201Ser	None Lunado (14)	L A	Thin	osomal dominant n hematuria	Lemmink et al., 1996
		Ser1238Stop	Juvenile (18)	1	+ g	arents, female patient tion, female patient	Mochizuki et al., 1994 Mochizuki et al. 1994

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(Boye et al., 1995). It is unclear where the missing mutations have to be found. Either they are located in the noncoding regions or in another Xlinked gene, or the patients do not suffer from Alport syndrome. Seven of the 38 large rearrangements (18%) and 14 of the 138 small mutations were de novo events (10%). Previously, it has been reported that in 15-18% of the cases no family history of Alport syndrome was present (Tryggvason et al., 1993), but this number included both de novo mutations and single autosomal recessive cases (Yoshikawa et al., 1987; Atkin et al., 1988). The large rearrangements may be due to misalignment of repeat sequences in the large COL4A5 and COL4A6 introns (Dahan et al., 1995). A percentage of 10–15% new mutations is comparable with other X-linked disorders.

#### EFFECT OF COL4A3, COL4A4, AND COL4A5 MUTATIONS

#### Amino acid substitutions

In the collagenous domain of the COL4A5 and COL4A4 genes the vast majority of the missense mutations in Alport syndrome replace glycine residues, which interrupt the characteristic triple Gly-X-Y repeat and impairs correct alignment of the type IV collagen triple helix. In familial benign hematuria, heterozygosity for a glycine substitution was identified as well. Because of the similarities with the other homozygous COL4A4 glycine substitution in an Alport patient, we hypothesized that familial benign hematuria could reflect manifesting carriership of autosomal recessive Alport syndrome (Lemmink et al., 1996), although this was not described for the parents of the Alport patient (Mochizuki et al., 1994). Of the remaining 11 cases, in which no glycine was involved, a cysteine residue was altered or created in three cases (Table 2). Cysteine residues are thought to play a role in intra- and interchain assembly of type IV collagen chains by disulfide bridge formation and substitutions can impair correct folding (Zhou et al., 1991b). The remainder of the amino acid substitutions are located in the NC domain and involve conserved amino acid residues. It is unclear whether the considered nonpathogenic amino acid substitutions have no ef-

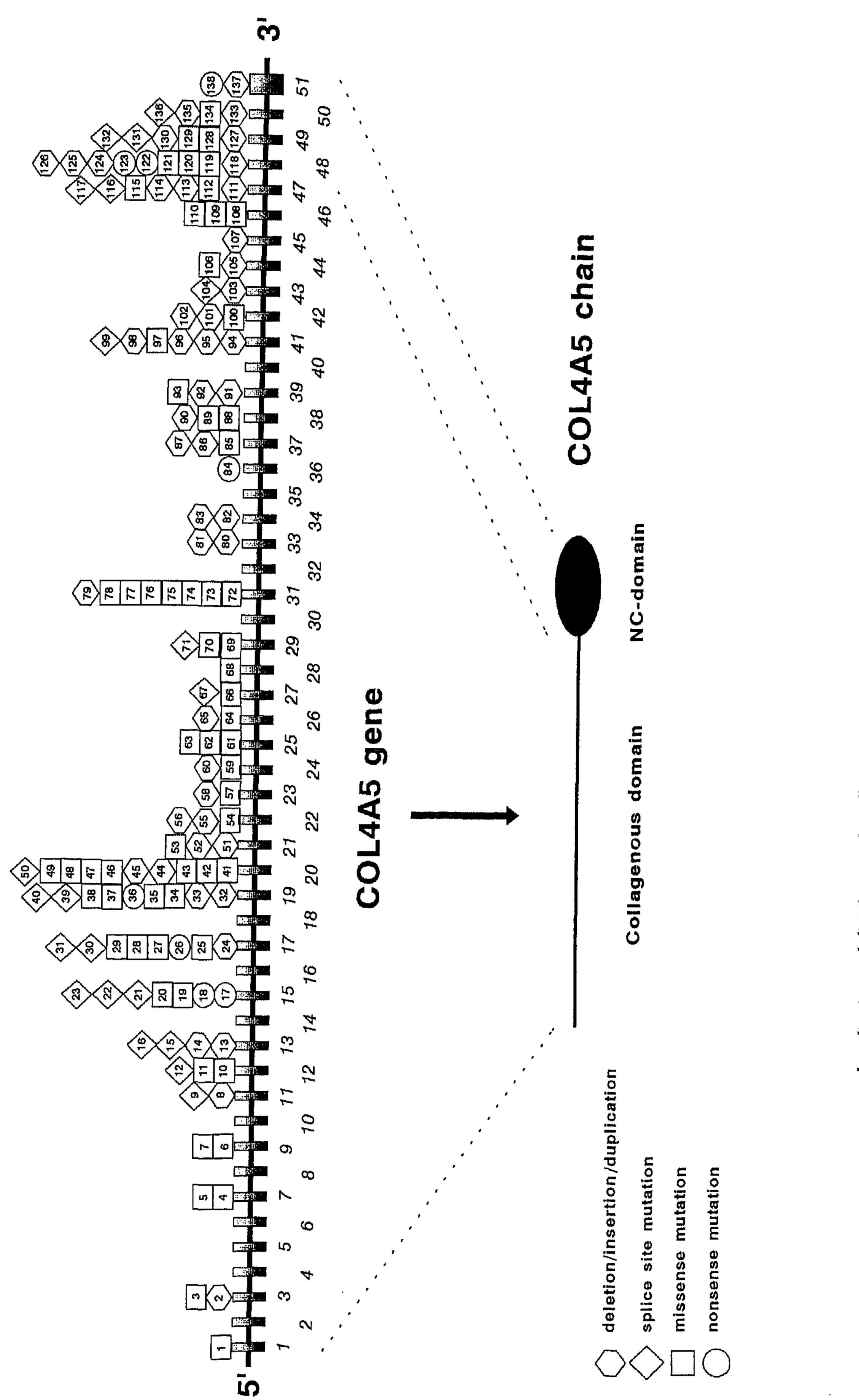
#### Distribution

The large deletions are dispersed all over the gene, and no hot spots have been identified. The number of small mutations seems to be less at the 5' end (Fig. 2), but most investigators have started their analysis from the 3' end. As yet, there is no evidence for mutational hot spots. Only a few mutations have been found more than once in different families. Substitutions of glycine residues at the 129, 325, 638, and 1143 positions were identified in nine families (Table 2). Each family revealed a different nucleotide substitution thereby excluding a common ancestor. Five different mutations were found in more than one unrelated family from the Netherlands, the United States, the United Kingdom, Finland, France, Japan, and Italy (Table 2). For example, the Arg1563 codon comprises the last 2 nucleotides of exon 48 and the first of exon 49. The one but last nucleotide of exon 48 (4889C $\alpha$ T) is altered in two families from the Netherlands and the United States (Zhou et al., 1993b) (Table 2), and the last nucleotide in exon 48 is changed in three unrelated families (4890G $\alpha$ A) from Denmark, the Netherlands, and the United States, respectively (Lemmink et al., 1993; Zhou et al., 1993b). In the Dutch family, we demonstrated that the 4890G $\alpha$ A originated in the mother of the patient (unpublished data). Haplotype analysis in Italian families using intragenic polymorphic markers indicated that the Gly869Arg and Gly866Glu mutations originated independently, while the 1685del34 mutation, present in two families, originated from a common ancestor (Renieri et al., 1996). The Gly869Arg was also found in a British family. Genealogical and haplotype studies of families with the same mutation are necessary to determine whether families are related.

fect at all. Probably they can modify other pathogenic COL4 mutations.

#### **Premature stopcodons**

The introduction of premature stop codons is predicted to lead to truncated proteins. However, such stop codons may impair the maturation of transcripts (Urlaub et al., 1989; Baserga and Benz, 1992) or alter the splicing of the pre-mRNA, resulting in skipping of the exon that contains the premature stopcodon (Dietz et al., 1993). In several studies on COL4A5 mRNA, the mutation was identified in the mRNA as such (Knebelmann et al., 1992; Lemmink et al., 1994a). However, only PBL RNA was studied, and a definite conclusion awaits the analysis of kidney samples from patients (Guo et al., 1993). In Samoyed dogs with Alport syndrome, kidney RNA has been tested (Zheng et al., 1994). The COL4A5 mutation in these dogs is Gly1027Stop. A drastic reduction of COL4A5 kidney mRNA was shown on Northern blot (Thorner et al., 1995). These observations are different from our observations in human PBL-RNA but, independent of the mechanism involved, the net result was always the absence of a functional COL4A5 chain.



correexons.

38, A5 C numbers individual Boxed indicate chain. 51  $\infty 5(IV)$ \*\*\*\* Numbers in italics, collagen the in ŝ ble 2 mutation in Tat small escribed of σ distribution mutations and gene Localization A5 Ö  $\bigcirc$ the 5 લં spond FIGURE

#### PHENOTYPE-GENOTYPE CORRELATIONS Leiomyomatosis (COL4A5/COL4A6 Mutations)

Patients suffering from Alport syndrome and diffuse esophageal and/or genital leiomyomatosis are characterized by having large deletions, extending from within the COL4A5 gene to intron 2 of the COL4A6 gene. The presumed absence of both the COL4A5 and COL4A6 chains prompted speculations about a role for type IV collagen in smooth muscle differentiation and morphogenesis (Zhou et al., 1993a). However, despite the deletion of the promoter sequence of the COL4A6 gene a specific, probably hybrid, COL4A6 transcript could be identified in esophageal tumour tissue of these patients (Heidet et al., 1995).

dred with an adult Alport phenotype and deafness (Renieri et al., 1994b), but a different substitution (Gly1143Asp) in a Danish kindred was associated with the juvenile phenotype without deafness (Zhou et al., 1992a). Identical amino acid substitutions cause different ages at onset of deafness (Barker et al., 1996). Of the six patients with a COL4A3 mutation, four were deaf and for the other two no auditory data were available. The two Alport patients with a COL4A4 mutation were not deaf. It has been speculated that the presence or absence of deafness could be related to tissue specific splicing (Brunner et al., 1991). Our data indicate that this is not the case. Mutations in the same exon can result in a phenotype with and without deafness. It seems more likely that the threshold for phenotypic effects differs between the kidney and the inner ear BM.

#### Juvenile and Adult Alport Syndrome (COL4A5 Mutations)

Mutations that are predicted to result in the complete absence of COL4A5 protein or truncated COL4A5 protein with no NC domain consistently cause the juvenile form of Alport syndrome. Because of the absence of the NC domains, the COL4A5 chain can not be incorporated into triple helical type IV collagen molecules. These mutations include all large rearrangements, but also those small mutations, that lead to premature stop codons. Only one of the latter mutations has the adult phenotype (Lemmink et al., 1993). Immunohistochemical studies have to reveal, if mutant COL4A5 chains of these patients are present in the GBM. Small inframe deletions or insertions cause juvenile Alport syndrome. Amino acid substitutions can lead to either juvenile or adult Alport syndrome. It is tempting to speculate that the juvenile form is predominantly associated with mutations resulting in absence of protein in the GBM and that the adult form may be caused by missense mutations that allow incorporation of the chain. This should be tested by immunohistochemistry. Finally, juvenile and adult Alport syndrome have been reported for members of the same family (Knebelmann et al., 1992; Renieri et al., 1994b), indicating that not only the specific mutations determines the phenotypic expression.

#### **Anti-GBM Nephritis**

About 1–5% of transplanted Alport patients develop a specific anti-GBM nephritis, subsequently leading to renal graft loss (Milliner et al., 1982). Data are available on 46 transplanted patients among whom were 41 with a COL4A5 mutation, four with a COL4A3 mutation and one with a COL4A4 mutation (Tables 1–3). All patients except one had juvenile Alport syndrome. In nine transplanted patients, 20% of the total number of transplantations, a specific anti-GBM nephritis was detected. All nine except one carried large deletions or premature stop codons and were predicted to result in truncated COL4A3 or COL4A5 proteins without NC domain. The one exception is a splice site mutation resulting in an mRNA without exon 38. The deletion does not disrupt the reading frame, but it should be tested whether this truncated chain is present in the GBM at all (Netzer et al., 1993). Four patients with characterized COL4A3 mutations were transplanted and three of them developed an anti-GBM nephritis (Table 3). These patients carry mutations which are predicted to produce COL4A3 protein from which most part of the NC domain is absent. These data strongly suggest that Alport patients with a type IV collagen mutation resulting in the absence of the NC domain have an increased risk of developing anti-GBM nephritis after renal transplantation (Smeets et al., 1992; Ding et al., 1995; this report).

#### Deafness (COL4A3/A4/A5 Mutations)

The majority of Alport patients suffer from progressive high-frequency sensorineural deafness. In the group of patients with COL4A5 mutations 30 male patients were not deaf of which four had adult and nine juvenile Alport syndrome. The other 17 patients were still too young to be included (Tables 1, 2). A Gly1143Ser mutation was identified in an Italian kin-

The absence of mutant  $\alpha(IV)$  chains can distort association with the other normal  $\alpha(IV)$  chains. The absence of normal and mutated type IV collagen chains in the GBM of patients corroborates with the finding that anti-GBM antibodies were directed against type IV collagen  $\alpha$ 3 and  $\alpha$ 5 epitopes, although not always at the mutated chain. A COL4A5 muta-

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tion can induce an anti-GBM nephritis either against COL4A3 or COL4A5 (Hudson et al., 1992; Kalluri et al., 1994; Kalluri et al., 1995). Anti-GBM antibodies in patients with COL4A3 mutations were directed against COL4A3 in one case and against both COL4A3 and COL4A5 chains in another case (Kalluri et al., 1995; van den Heuvel, unpublished data). It is obvious that other factors than type IV collagen defects are implicated in the immune response, as about 70% of Alport patients contain no  $\alpha 5$  (IV) protein in the GBM, only 1–5% of the transplanted Alport patients develop anti-GBM nephritis (Milliner et al., 1982; Gubler et al., 1993). A similar conclusion can be drawn from the absence of anti-GBM posttransplant nephritis in patients with deletions of either the COL4A3 or COL4A5 NC domain or the entire COL4A5 chain (Gubler et al., 1993), the absence of anti-GBM nephritis in a second graft (Milliner et al., 1982; Rassoul et al., 1990), and the intrafamilial discordance with regard to the occurrence of GBM alloimmunization, observed in one family with a COL4A5 deletion (Kashtan et al., 1990).

and transfection studies, will be employed to study the manifestations of the mutations at the RNA and protein level. The reported coordinated reduction of transcription of the COL4A3 and COL4A4 genes, because of a mutation in the COL4A5 gene, in a dog model (Thorner et al., 1996), has to be confirmed for mutations in humans. Furthermore, intrafamilial variation in expression of Alport syndrome with respect to ESRD and development of anti-GBM nephritis suggests that it is not only the genetic defect that counts, but also a yet unknown number of factors, which contribute to the nature and severity of clinical manifestations. The implications of type IV collagen defects have become of more general importance by the identification of a COL4A4 mutation in persistent hematuria and of an unexplained role in benign smooth muscle cell proliferation in leiomyomatosis, associated with Alport syndrome. Basic information on type IV collagen gene regulation, precise definition of all aspects of the genetic defects, and risk factors and knowledge of the different physiological roles of type IV collagen is of key importance for prognostic predictions and for the successful development of therapeutic approaches, such as gene therapy, which are currently being investigated (Tryggvason et al., in press).

#### **FUTURE PROSPECTS**

Progress in defining the type IV collagen gene mutations in Alport syndrome has been extremely rapid during the last couple of years. The number of mutations in the COL4A5 gene is yet sufficiently high for initial genotype--phenotype correlations. Still, only 50% of the mutations has been detected in evident X-linked Alport syndrome. Research will be directed at screening the non-coding areas of the COL4A5 gene and at the development of new technology for mutation detection to find the remainder of the mutations. The recently reported method of resequencing complex genes by high-density DNA arrays or "CHIPs" (Chee et al., 1996) is very promising in this respect. On the other hand, comparable with hemophilia A (Lakich et al., 1993), a major mutation may long be uncharacterized and can exist also in X-linked Alport syndrome. Apart from new molecular technology, a comprehensive protocol has to be developed based on clinical records, family history, and immunohistochemistry with chain-specific antibodies to guide mutation analysis in the three huge collagen genes. Especially, immunohistochemical analysis of the epidermal basement membrane (EBM) is promising as a first test, as about 70% of the patients with a mutation in the COL4A5 gene lack this chain from the EBM (Zhou and Reeders, 1996). Many of the functional consequences of type IV collagen mutations are, however, based on predictions rather than on experimental evidence. Experiments like in situ hybridization, immunohistochemistry,

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#### REFERENCES

- Alport AC (1927) Hereditary familial congenital haemorrhagic nephritis. BMJ 1:504-506.
- Antignac C, Zhou J, Sanak M, Cochat P, Roussel B, Deschenes G, Knebelmann B, Hors-Cayla MC, Tryggvason K, Gubler M-C (1992) Alport syndrome and diffuse leiomyomatosis: Deletions in the 5<sup>-</sup>

end of the COL4A5 collagen gene. Kidney Int 42:1178–1183.

Antignac C, Knebelmann B, Drouot L, Gros F, Deschenes G, Hors-Cayla MC, Zhou J, Tryggvason K, Grunfeld JP, Broyer M, Gubler MC (1994) Deletions in the COL4A5 collagen gene in X-linked Alport syndrome. J Clin Invest 93:1195–1207.

Atkin CL, Hasstedt SJ, Menlove L, Cannon L, Kirschner N, Schwartz C, Nguyen K, Skolnick MH (1988) Mapping of Algort syndrome to the long arm of the X chromosome. Am J Hum Genet 42:249–255.

- Barker D, Hostikka SL, Zhou J, Chow T, Oliphant AR, Gerken SC, Gregory MC, Skolnick MH, Atkin CL, Tryggvason K (1990) Identification of mutations in the COL4A5 collagen gene in Alport syndrome. Science 248:1224-1227.
- Barker DF, Pruchno CJ, Jiang X, Atkin CL, Stone EM, Denison 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.
- Baserga SJ, Benz EJ Jr (1992) Beta-globin nonsense mutation: Deficient accumulation of mRNA occurs despite normal cytoplasmic stability. Proc Natl Acad Sci USA 89:2935-2939.
- Beaudet AL, Tsui L-C (1993) A suggested nomenclature for designating mutations. Hum Mutat 2:245–248. Bernal D, Quinones S, Saus J (1993) The human mRNA encoding the Goodpasture antigen is alternatively spliced. J Biol Chem 268:12090–12094.

gene in a woman with Alport syndrome and posttransplant antiglomerular basement membrane nephritis. J Am Soc Nephrol 5:1714–1717.

- Epstein CJ, Sahud MA, Piel CF, Goodman JR, Bernafield MR, Kushner IH, Ablin AR (1972) Hereditary macrothrombocytopenia, nephritis and deafness. Am J Med 52:299–310.
- Flinter FA (1993) Molecular genetics of Alport's syndrome (1993) Q I Med 86:289–292.
- Flinter FA, Cameron JS, Chantler C, Houston I, Bobrow M (1988) Genetics of classic Alport syndrome. Lancet 2:1005–1007.
- Flinter FA, Abbs S, Bobrow M (1989) Localization of the gene for classic Alport syndrome. Genomics 4:335–338.
- Garcia-Torres R, Orozco L (1993) Alport-leiomyomatosis syndrome: An update. Am J Kidney Dis 22:641–648.
- Boyd CD, Wiliky K, Toth-Fejel SE, Deal SB, Christiano AM, Mackenzie JW, Sandell LJ, Tryggvason K, Magenis E (1986) The single copy gene coding for human  $\alpha 1$  (IV) Procollagen is located at the terminal end of the long arm of chromosome 13. Hum Genet 74:121–125.
- Boye E, Vetrie D, Flinter F, Buckle B, Pihlajaniemi T, Hamalainen ER, Myers JC, Bobrow M, Harris A (1991) Major rearrangements in the  $\alpha$ 5(IV) collagen gene in three patients with Alport syndrome. Genomics 11:1125-1132.
- Boye E, Flinter FA, Bobrow M, Harris A (1993) An 8 bp deletion in exon 51 of the COL4A5 gene of an Alport syndrome patient. Hum Mol Genet 2:595–596.
- Boye E, Flinter F, Zhou J, Tryggvason K, Bobrow M, Harris A (1995) Detection of 12 novel mutations in the collagenous domain of the COL4A5 gene in Alport patients. Hum Mutat 5:197-204.
- Brunner HG, Schröder CH, van Bennekom C, Lambermon E, Tuerlings J. Menzel D. Olbing H. Monnens LAH, Wieringa B. Ropers HH (1988) Localization of the gene for X-linked Alport's syndrome. Kidney Int 34:507–510. Brunner HG, Smeets B, Smeets D, Nelen M, Cremers CWRJ, Ropers H-H (1991) Molecular genetics of X-linked hearing impairment. Ann NY Acad Sci 630:176–190.

- Gauthier B, Trachtmann H, Frank R, Valderrama E (1989) Familial thin basement membrane nephropathy in children with asymptomatic microhematuria. Nephron 51:502–508.
- Gubler MC, Gonzales-Burchard G, Monnier C (1976) Alport's syndrome: natural history and ultrastructural lesions of glomerular and tubular basement membranes. Contrib Nephrol 2:163–169.
- Gubler MC, Antignac C, Deschenes G, Knebelmann B, Hors-Cayla MC, Grunfeld JP, Broyer M, Habib R (1993) Genetic, clinical and morphological heterogeneity in Alport syndrome. Adv Nephrol 22:15–35.
- Guo C, Van Damme B, Van Damme-Lombaerts R, Van Den Berghe H, Cassiman J-J, Marynen P (1993) Differential splicing of COL4A5 mRNA in kidney and white blood cells: A complex mutation in the COL4A5 gene of an Alport patient deletes the NC1 domain. Kidney Int 44:1316–1321.
- Guo C, Van Damme B, Vantererghem 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.
- Hamalainen E-R, Renieri A, Pecoraro C, De Marchi M, Pihlajaniemi
- Cameron JS (1991) Recurrent primary disease and de novo nephritis following renal transplantation. Pediatr Nephrol 5:412–421.
- Chan B, Antignac C, Gubler MC, Deschênes G, Mariyama M, Mochizuki M, Reeders ST (1993) A new locus for Alport syndrome: Linkage of autosomal recessive Alport syndrome to the gene encoding the  $\alpha$ 3 chain of type IV collagen. In Proceedings of the Second International Workshop on Alport Syndrome, New Haven, p 3.
- Chee M, Yang R, Hubbell E, Berno A, Huang XC, Stern D, Winkler J, Lockhart DJ, Morris MS, Fodor SPA (1996) Accessing genetic information with high-density DNA arrays. Science 274:610-614.
- Cochat P, Guibaud P, Torres R, Roussel B, Guarner V, Larbre F (1988) Diffuse leiomyomatosis in Alport syndrome. J Pediatr 113:339-343.
- Dahan K, Heidet L, Zhou J, Mettler G, Leppig KA, Proesmans W, David A, Roussel B, Mongeau JG, Gould JMD, Grunfeld J-P, Gubler M-C, Antignac C (1995) Smooth muscle tumors associated with X-linked Alport syndrome: Carrier detection in females. Kidney Int 48:1900–1906.

- T (1996) Unequal homologous crossing over resulting in duplication of 36 basepairs with exon 47 of the COL4A5 gene in family with Alport syndrome. Hum Mutat 8:265–269.
- Hasstedt SJ, Atkin CL, San Juan AC (1986) Genetic heterogeneity among kindreds with Alport syndrome. Am J Hum Genet 38:940-953.
- Heidet L, Dahan K, Zhou J, Xu Z, Cochat P, Gould JDM, Leppig KA, Proesmans W, Guyot C, Roussel B, Tryggvason K, Grunfeld JP, Gubler MC, Antignac C (1995) Deletions of both  $\alpha$ 5(IV) and  $\alpha 6(IV)$  collagen genes in Alport syndrome and in Alport syndrome associated with smooth muscle tumours. Hum Mol Genet 4:99-108.
- Heiskari N, Zhang X, Zhou J, Leinonen A, Barker D, Gregory M, Atkin CL, Netzer KO, Weber M, Reeders S, Gronhagenriska 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.
- Hertz JM, Heiskari N, Zhou J, Jensen UB, Tryggvason K (1995) A nonsense mutation in the COL4A5 collagen gene in a family with X-linked juvenile Alport syndrome. Kidney Int 47:327-332.

Dietz HC, Valle D, Francomano CA, Kendzior RJ, Pyeritz RE, Cutting GR (1993) Skipping of constitutive exons in vivo induced by nonsense mutations. Science 259:680-683.

Ding J, Zhou J, Tryggvason K, Kashtan CE (1994) COL4A5 deletions in three patients with Alport syndrome and posttransplant antiglomerular basement membrane nephritis. J Am Soc Nephrol 5:161-168.

Ding J, Stitzel J, Berry P, Hawkins E, Kashtan CE (1995) Autosomal recessive Alport syndrome: Mutation in the COL4A3

Hostikka SL, Tryggvason K (1987) Extensive structural differences between genes for the  $\alpha 1$  and  $\alpha 2$  chains of type IV collagen despite conservation of coding sequences. FEBS Lett 224:297–305.

Hostikka SL, Eddy RL, Byers MG, Höyhtya M, Shows TB, Tryggvason K (1990) Identification of a distinct type IV collagen  $\alpha$  chain with restricted kidney distribution and assignment to the locus of the X chromosome-linked Alport syndrome. Proc Natl Acad Sci USA 87:1606–1610.

Hudson BG, Kalluri R, Gunwar S, Weber M, Ballester F, Hudson JK,

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Noelken ME, Sarras M, Richardson WR, Saus J, Abrahamson DR, Glick AD, Haralson MA, Helderman JH, Stone WJ, Jacobson HR (1992) The pathogenesis of Alport syndrome involves type IV collagen molecules containing the  $\alpha 3$ (IV) chain: Evidence from anti-GBM nephritis after renal transplantation. Kidney Int 42:179–187.

- Hudson BG, Reeders ST, Tryggvason K (1993) Type IV collagen: Structure, gene organization, and role in human diseases. J Biol Chem 268:26033-26036.
- Jefferson JA, Lemmink HH, Hughes AE, Hill CM, Smeets HJM, Doherty CC, Maxwell AP (1997) Autosomal dominant Alport syndrome linked to the type IV collagen  $\alpha$ 3 and  $\alpha$ 4 genes (COL4A3 and COL4A4). (submitted)
- Kalluri R, Gunwar S, Reeders ST, Morrison KE, Mariyama M, Ebner KE, Noelken ME, Hudson BG (1991) Goodpasture syndrome: Localization of the epitope for the autoantibodies to the carboxylterminal region of the α3(IV) chain of basement membrane collagen. J Biol Chem 266:24018–24024.

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.

Krawczak M, Reiss J, Cooper DN (1992) The mutational spectrum of single base-pair substitutions in mRNA splice junctions of human genes: Causes and consequences. Hum Genet 90:41-54.

Kühn K (1994) Basement membrane (type IV) collagen. Matrix Biol 14:439–445.

Lakich D, Kazazian HH, Antonarakis SE, Gitschier J (1993) Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nature Genet 5:236-241.

Leinonen A, Mariyama M, Mochizuki T, Tryggvason K, Reeders ST

- 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.
- Kalluri R, Van den Heuvel LP, Smeets HJM, Schröder CH, Lemmink HH, Boutaud A, Neilson EG, Hudson BG (1995) A COL4A3 gene mutation and post-transplant anti-α3(IV) collagen alloantibodies in Alport syndrome. Kidney Int 47:1199–1204.
- Kamagata Y, Mattei M-G, Ninomiya Y (1992) Isolation and sequencing of cDNAs and genomic DNAs encoding the α4 chain of basement membrane collagen type IV and assignment of the gene to the distal long arm of human chromosome 2. J Biol Chem 267:23753-23758.
- Kashtan CE, Butkowski RJ, Kleppel MM, Roy First M, Michael AF (1990) Posttransplant anti-glomerular basement membrane nephritis in related males with Alport syndrome. J Lab Clin Med 116:508–515.
- Kawai S, Nomura S, Harano T, Harano K, Fukushima T, Osawa G, and the Japanese Alport network (1996) The COL4A5 gene in Japanese Alport syndrome patients: Spectrum of mutations of all exons. Kidney Int 49:814–822.

- (1994) Complete primary structure of the human type IV collagen α4(IV) chain. J Biol Chem 269:26172-26177.
- Lemmink HH, Schröder CH, Brunner HG, Nelen MR, Zhou J, Tryggvason K, Haagsma-Schouten WAG, Roodvoets AP, Rascher W, van Oost BA, Smeets HJM (1993) Identification of four novel mutations in the COL4A5 gene in patients with Alport syndrome. Genomics 17:485–489.
- Lemmink HH, Kluijtmans LAJ, Brunner HG, Schröder CH, Knebelmann B, Jelinkova E, van Oost BA, Smeets HJM (1994a) Aberrant splicing of the COL4A5 gene in patients with Alport syndrome. Hum Mol Genet 3:317–322.
- Lemmink HH, Mochizuki T, van den Heuvel LPWJ, Schröder CH, Barrientos A, Monnens LAH, van Oost, Brunner HG, Reeders ST, Smeets HJM (1994b) Mutations in the type IV α3 (COL4A3) gene in autosomal recessive Alport synchrome. Hum Mol Genet 3:1269–1273.
- Lemmink HH, Nillesen WN, Mochizuki T, Schröder CH, Brunner HG, van Oost BA, Monnens LAH, Smeet: HJM (1996) Benign familial hematuria due to mutation of the type IV collagen α4 gene. J Clin Invest (in press).
- Mariyama M, Zheng K, Yang-Feng TL, Reeders S (1992) Colocalization of the genes for the α3(IV) and α4(IV) chains of type IV collagen to chromosome 2 bands q35-37. Genomics 13:809-813.
- Kitagawa K, Nakanishi K, Lijima K, Nishio H, Sano K, Nakamura H, Yoshikawa N (1995) Mutation in α5(IV) collagen chain gene in nonfamilial hematuria. J Am Soc Nephrol 6:264–268.
- Kleppel MM, Michael AF (1990) Expression of novel basement membrane components in developing human kidney and eye. Am J Anat 187:165–174.
- 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.
- Kleppel M, Santi PA, Cameron JD, Wieslander J, Michaels AF (1989a) Human tissue distribution of novel basement membrane collagen. Am J Pathol 134:813–825.
- Kleppel MM, Kashtan C, Santi PA, Wieslander J, Michael AF (1989b) Distribution of familial nephritis antigen in normal tissue and renal basement membranes of patients with homozygous and heterozygous Alport familial nephritis. Lab Invest 61:278-289.
- Kleppel MM, Fan WW, Cheong HI, Michael AF (1992) Evidence for

- Mariyama M, Leinonen A, Mochizuki T, Tryggvason K, Reeders ST (1994) Complete primary structure of the human α3(IV) collagen chain. Coexpression of the α3(IV) and α4(IV) collagen chains in human tissues. J Biol Chem 269:23013-23017.
- Massella L, Rizzoni G, De Blasis R, Barsotti P, Farraggiana, Renieri A, Seri M, Galli L, DeMarchi M (1994) De novo COL4A5 gene mutations in Alport's syndrome. Nephrol Dial Transplant 9:1408– 1411.
- Milliner DS, Pieredes AN, Holley KE (1982) Renal transplantation in Alport's syndrome: anti-glomerular basement membrane glomerulonephritis in the allograft. Mayo Clin Proc 57:35-43.
- 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.
- Mochizuki T, Lemmink HH, Mariyama M, Antignac C, Gubler M-C, Pirson Y, Verellen-Dumoulin C, Schröder CH, Smeets HJM, Reeders ST (1994) Identification of mutations in the α3(IV) and α4(IV) collagen genes in autosomal recessive Alport syndrome. Nature Genet 8:77–82.
- Nakazato H, Hattori S, Matsuura T, Koitabashi Y, Endo F, Matsuda I (1993) Identification of a single base insertion in the COL4A5

separate networks of classical and novel basement membrane collagen, Characterization of α3(IV) Alport antigen heterodimer. J Biol Chem 267:4137-4142.

Knebelmann B, Deschenes G, Gros F, Hors M-C, Grunfeld JP, Zhou J, Tryggvason K, Gubler M-C, Antignac C (1992) Substitution of arginine for glycine 325 in the collagen α5(IV) chain associated with X-linked Alport syndrome: Characterization of the mutation by direct sequencing of PCR-amplified lymphoblast cDNA fragments. Am J Hum Genet 51:135–142. gene in Alport syndrome. Kidney Int 44:1091–1096.

Nakazato H, Hattori S, Ushijima T, Matsuura T, Koitabashi Y, Takada T, Yoshioka K, Endo F, Matsuda I (1994) Mutations in the COL4A5 gene in Alport syndrome: A possible mutation in primordial cells. Kidney Int 46:1307–1314.

Nakazato H, Hattori S, Ushijima T, Matsuura S, Karashima S, Uemura T, Endo F, Matsuda I (1995) Splicing mutations in the COL4A5 gene in Alport's syndrome: Differential mRNA expression between leukocytes and fibroblasts. Am J Kidney Dis 26:732-739.

- Netzer KO, Renders L, Zhou J, Pullig O, Tryggvason K, Weber M (1992) Deletions of the COL4A5 gene in patients with Alport syndrome. Kidney Int 42:1336-1344.
- Netzer KO, Pullig O, Zhou J, Frei U, Tryggvason K, Weber M (1993) COL4A5 splice site mutation and α5(IV) collagen mRNA in Alport syndrome. Kidney Int 43:486-492.
- Ninomiya Y, Kagawa M, Iyama K-I, Naito I, Kishoro 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.
- Nomura S, Osawa G, Sai T, Harano T, Harano K (1993) A splicing mutation in the α5(IV) collagen gene of a family with Alport syndrome. Kidney Int 43:1116–1124.

Renieri A, Galli L, Grillo A, Bruttini M, Neri T, Zanelli P, Rizzoni G, Masella L, Sessa A, Meroni M, Peratoner L, Riegler P, Scolari F, Mileti M, Giani M, Cossu M, Saci M, Ballabio M, DeMarchi M (1995) Major COL4A5 gene rearrangements in patients with juvenile Alport syndrome. Am J Med Genet 59:380–385.

- Renieri A, Bruttini M, Galli L, Zanelli P, Neri T, Rosetti S, Turco A, Heiskari N, Zhou J, Gusmano R, Masella L, Bampi G, Scolari F, Sessa A, Rizzoni G, Tryggvason K, Pignatti PF, Saci M, Ballabio A, DeMarchi M (1996) X-linked Alport syndrome: An SSCP based mutation survey over all 51 exons of the COL4A5 gene. Am J Hum Genet 58:1192–1204.
- Saito A, Sakatsume, Yamazaki H, Ogata F, Hirasawa Y, Arakawa M (1994) A deletion in the 3<sup>-</sup> end of the α5(IV) collagen gene in juvenile-onset Alport syndrome. J Am Soc Nephrol 4:1649–1653.
- Sanes JR, Engvall E, Butkowski R, Hunter DD (1990) Molecular heterogeneity of basal laminae: Isoforms of laminin and collagen IV at neuromuscular junctions and elsewhere. J Cell Biol 111:1685-1699.
- Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879.
- Peissel B, Rossetti S, Renieri A, Galli L, De Marchi M, Battini G, Meroni M, Sessa A, Schiavano S, Pignatti PF, Turco AE (1994) A novel frameshift deletion in type IV collagen α5 gene in a juvenile-type Alport syndrome patient: an adenine deletion (24940/ 2943 del A) in exon 34 of COL4A5. Hum Mutat 3:386–390.
- Perrin D, Jungers P, Grunfeld J-P, Delons S, Noël LH, Zenatti C (1980) Perimacular changes in Alport's syndrome. Clin Nephrol 13:163–167.
- Peterson LC, Rao VK, Crosson JT, White JG (1985) Fechtner syndrome—A variant of Alport's syndrome with leukocyte inclusions and macrothrombocytopenia. Blood 65:397-406.
- Pihlajaniemi T, Tryggvason K, Myers J, Kurkinen M, Lebo R, Cheung M, Prockop DJ, Boyd CD (1985) cDNA clones coding for the pro-α-1(IV) chain of human type IV procollagen reveal an unusual homology of amino acid sequences in two halves of the carboxyl-terminal domain. J Biol Chem 260:7681–7687.
- Quinones S, Bernal D, Garcia-Sogo M, Elena SF, Saus J (1992) Exon/ intron structure of the human α3(IV) gene encompassing the Goodpasture antigen (α3(IV)NC1). Identification of a potentially antigenic region at the triple/NC1 domain junction. J Biol Chem 267:19780–19784.

- Savage COS, Noel LH, Crutcher E, Price SRG, Grunfeld JP, Lockwood CM (1989) Hereditary nephritis: Immunoblotting studies of the glomerular basement membrane. Lab Invest 60:613–618.
- Siebold B, Deutzmann R, Kuhn K (1988) The arrangement of intraand intermolecular disulfide bonds in the carboxyterminal, noncollagenous aggregation and cross-linking domain of basement-membrane type IV collagen. Eur J Biochem 176:617-624.
- Smeets HJM, Melenhorst JJ, Lemmink HH, Schröder CH, Nelen MR, Zhou J, Hostikka SL, Tryggvason K, Ropers H-H, Jansweijer MCE, Monnens LAH, Brunner HG, van Oost BA (1992) Different mutations in the COL4A5 gene in two patients leading to different types of Alport syndrome. Kidney Int 42:83–88.
- Sohar E (1956) Renal disease, inner ear deafness and ocular changes. Arch Intern Med 97:627-630.
- Soininen R, Huotari M, Ganguly A, Prockop DJ, Tryggvason K (1989) Structural organization of the gene for the α1 chain of human type IV collagen. J Biol Chem 264:13565–13571.
- Spear GS, Slusser RJ (1972) Alport's syndrome: Emphasizing electron microscopic studies of the glomerulus. Am J Pathol 69:213–222.
- Rassoul Z, Al-khader AA, Al-Sulaiman M, Dhar JM, Coode P (1990) Recurrent allograft antiglomerular basement membrane glomerulonephritis in a patient with Alport's syndrome. Am J Nephrol 10:73–76.
- Renieri A, Seri M, Myers JC, Pihlajaneimi T, Massela L, Rizzoni G, De Marchi M (1992a) De novo mutation in the COL4A5 gene converting glycine 325 to glutamic acid in Alport syndrome. Hum Mol Genet 1:127–129.
- Renieri A, Seri M, Myers JC, Pihlajaniemi T, Sessa A, Rizzoni G, De Marchi M (1992b) Alport syndrome caused by a 5<sup>-7</sup> deletion within the COL4A5 gene. Hum Genet 89:120–121.
- Renieri A, Seri M, Galli L, Cosci P, Imbasciati E, Massella L, Rizzoni G, Restagno G, Carbonara AO, Stramignoni E, Basolo B, Piccoli G, De Marchi M (1993) Small frameshift deletions within the COL4A5 gene in juvenile-onset Alport syndrome. Hum Genet 92:417–420.
- Renieri A, Galli L, De Marchi M, Li Volti S, Mollica F, Peissel B, Pignatti P, Turco A (1994a) Single base pair deletions in exons 39 and 42 of the COL4A5 gene in Alport syndrome. Hum Mol Genet 3:201–202.

- Sugimoto M, Oohashi T, Yoshioka H, Matsuo N, Ninomiya Y (1993) cDNA isolation and partial gene structure of the human α4(IV) collagen chain. FEBS Lett 330:122–128.
- Sugimoto M, Oohashi T, Ninomiya Y (1994) The genes COL4A5 and COL4A6, coding for basement membrane collagen chains α5(IV) and α6(IV), are located head-to-head in close proximity on human chromosome Xq22 and COL4A6 is transcribed from two alternative promoters. Proc Natl Acad Sci USA 91:11679–11683.
- Thorner P, Baumal R, Eddy A, Marrano PM (1990) A study by immunofluorescence of the NC1 domain of collagen type IV in glomerular basement membranes of two patients with hereditary nephritis. Virchow Arch A Pathol Anat 416:205–212.
- Thorner P, Zheng K, Jacobs R, Kalluri R, Hudson BG (1995) X-linked hereditary nephritis in samoyed dogs: A model for human X-linked hereditary nephritis. In the Seventh International Symposium on Basement Membranes, NIH Bethesda, June 29–30, p. 140.
- 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 71:13821–13828.

Timpl R (1989) Structure and biological activity of basement membrane proteins. Eur J Biochem 180:487–502.

Renieri A, Meroni M, Sessa A, Battini G, Serbelloni P, Torri Tarelli L, Seri M, Galli L, De Marchi M (1994b) Variability of clinical phenotype in a large Alport family with gly1143ser change of collagen α5(IV) chain. Nephron 67:444–449.

Renieri A, Bassi MT, Galli L, Zhou J, Giani M, De Marchi M, Ballabio A (1994c) Deletion spanning the 5' ends of both the COL4A5 and COL4A6 genes in a patient with Alport's syndrome and leiomyomatosis. Hum Mutat 4:195–198. Tryggvason K, Heikillä P, Petterson E, Tibell A, Thorner P (1997) Can Alport syndrome be treated by gene therapy? Kidney Int (in press).

Tryggvason K, Zhou J, Hostikka SL, Shows T (1993) Molecular genetics of Alport syndrome. Kidney Int 43:38–44.

Turco AE, Rossetti S, Biasi MO, Rizzoni G, Massella L, Saarinen NH,

#### CLINICAL SPECTRUM OF TYPE IV COLLAGEN MUTATIONS 499

- Renieri A, Pignatti FP, De Marchi M (1995) A novel missense mutation in exon 3 of the COL4A5 gene associated with lateonset Alport syndrome. Clin Genet 48:261–263.
- Tverskaya S, Bobrynina V, Tsalykova F, Ignatova M, Krasnopolskaya X, Evgrafov O (1996) Substitution of A1498D in noncollagen domain of α 5(IV) collagen chain associated with adult-onset X-linked Alport syndrome. Hum Mutat 7:149–150.
- Urlaub G, Mitchell PJ, Ciudad CJ, Chasin LA (1989) Nonsense mutations in the hydrofolate reductase gene affect RNA processing. Mol Cell Biol 9:2868–2880.
- Van den Heuvel LPWJ, Schröder CH, Savage COS, Menzel D, Assmann KJM, Monnens LAH, Veerkamp JH (1989) The development of anti-glomerular basement membrane nephritis in two
- Zheng K, Thorner PS, Marrano P, Baumal R, McInnes RR (1994) Canine X chromosome-linked hereditary nephritis: A genetic model for human X-linked hereditary nephritis resulting from a single base mutation in the gene encoding the α5 chain of collager, type IV. Proc Natl Acad Sci USA 91:3989–3993.
- Zhou J, Reeders ST (1996) The α chains of type IV collagen. In Tryggvason K (ed): Contributions to Nephrology. Vol. 117: Molecular Pathology and Genetics of Alport Syndrome. Basel: S. Karger, pp 80–105.
- Zhou J, Hostikka SL, Chow LT, Tryggvason K (1991a) Characterization of the 3' half of the human type IV collagen α5 gene which is affected in Alport syndrome. Genomics 9:1–9.
- Zhou J, Barker D, Hostikka SL, Gregory M, Atkin C, Tryggvason K (1991b) Single base mutation in α5(IV) collagen chain gene converting a conserved cysteine to serine in Alport syndrome. Genomics 9:10–18.

children with Alport's syndrome: Characterization of the antibody target. Pediatr Nephrol 3:406–413.

- Vetrie D, Boye E, Flinter F, Bobrow M, Harris A (1992) DNA rearrangements in the α5(IV) collagen gene (COL4A5) of individuals with Alport syndrome: Further refinement using pulse-field gel electrophoresis. Genomics 14:624–633.
- Yamazaki H, Nakagawa Y, Saito A, Nishi S, Sakatsume M, Takeda T, Maruyama Y, Ogino S, Maruyama S, Machizuki T, Reeders ST, Arakawa M (1995) No linkage to the COL4A3 gene locus in Japanese thin basement membrane disease families. Nephrol 1:315–321.
- Yoshikawa N, Matsuyama S, Ito H, Hajikano H, Matsuo T (1987) Nonfamilial hematuria associated with glomerular basement membrane alterations characteristic of hereditary nephritis: comparison with hereditary nephritis. J Pediatr 111:519–524.
- Yoshioka K, Hino S, Takemura T, Maki S, Wieslander J, Takekoshi Y, Makino H, Kagawa M, Sado Y, Kashtan CE (1994) Type IV collagen  $\alpha$ 5 chain. Normal distribution and abnormalities in X-linked Alport syndrome revealed by monoclonal antibody. Am J Pathol 144:986–996.
- Yurchenco PD, Ruben GC (1987) Basement membrane structure in situ: Evidence for lateral associations in the type IV collagen net-

- Zhou J, Hertz JM, Tryggvason K (1992a) Mutation in the α5(IV) collagen chain in juvenile-onset Alport syndrome without hearing loss or ocular lesions: Detection by denaturing gradient gel electrophoresis of PCR product. Am J Hum Genet 50:1291–1300.
- Zhou J, Hertz JM, Leinonen A, Tryggvason K (1992b) Complete amino acid sequence of the human α5(IV) collagen chain and identification of a single base mutation in exon 23 converting glycine-521 in the collagenous domain to cysteine in an Alport syndrome patient. J Biol Chem 267:12475–12481.
- Zhou J, Mochizuki T, Smeets H, Antignac C, Laurila P, de Paepe A, Tryggvason K, Reeders ST (1993a) Deletion of the paired α5(IV) and α6(IV) collagen genes in inherited smooth muscle tumors. Science 261:1167–1169.
- Zhou J, Gregory MC, Hertz JM, Barker DF, Atkin C, Spencer E, Tryggvason K (1993b) Mutations in the codon for a conserved arginine-1563 in the COL4A5 collagen gene in three unrelated families with juvenile type classical Alport syndrome. Kidney Int 43:722–729.
- Zhou J, Leinonen A, Tryggvason K (1994a) Structure of the human type IV collagen COL4A5 gene. J Biol Chem 269:6608–6614.

work. J Cell Biol 105:2559–2568.

- Zhang X, Zhou J, Reeders ST, Tryggvason K (1996) Structure of the human type IV collagen COL4A6 gene, which is mutated in Alport syndrome-associated leiomyomatosis. Genomics 33:473–479.
- Zhou J, Ding M, Zhao Z, Reeders ST (1994b) Complete primary structure of the sixth chain of human basement membrane collagen, α6(IV). J Biol Chem 269:13193–13199.