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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.

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

STRUCTURE AND FUNCTION OF TYPE IV COLLAGEN

Type IV collagen is a multimeric protein composed of three so-called α chains. To date, six different α chains have been identified (α1–α6) with molecular weights of 170–185 kDa. Each α(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).

The six α(IV) chains interact in different tissues in different combinations. The [α1]2α2 (IV) and [α1]3(IV) hetero- and homotrimers are ubiquitously present in all basement membranes (Hudson et al., 1993a). The α3(IV), α4(IV), α5(IV), and α6(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 α1 and α2 heterotrimers and one of a tissue-specific combination of the other chains (Kleppel et al., 1992; Hudson et al., 1993). For example, the α1(IV) and α2(IV) chains reside in the subendothelial layer of the glomerular basement membrane (GBM), while the α3(IV), α4(IV), and α5(IV) chains are particularly prominent in the lamina densa (Kleppel et al., 1989a) (Fig. 1). The high cysteine content of the α4(IV) chain, probably involved in intermolecular assembly, may result in additional strength to the GBM. The α3(IV) and α4(IV) chains colocalize in a large set of basement

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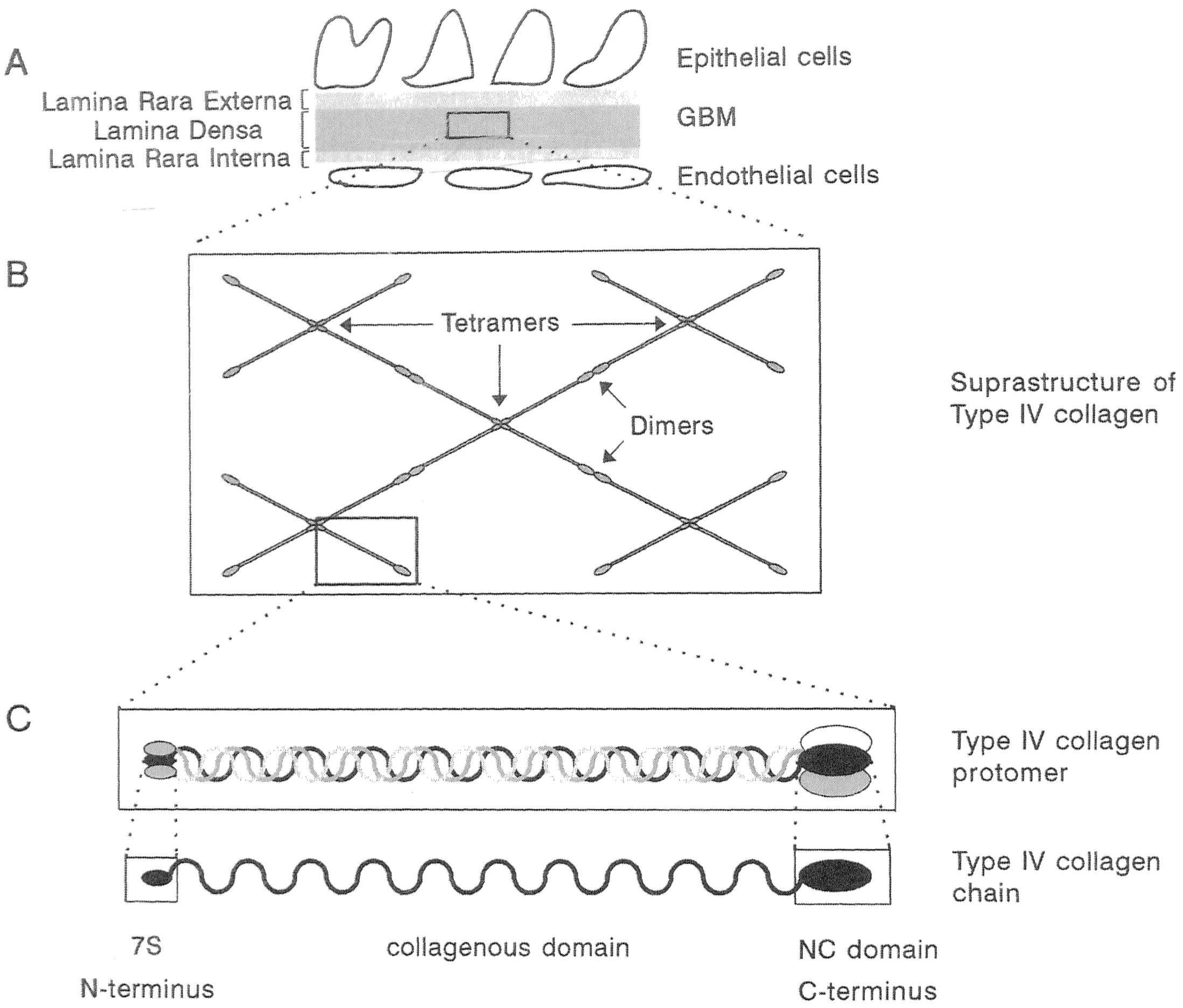

FIGURE 1. Schematic representation of the molecular structure of type IV collagen in renal glomerular basement membrane. **A:** The glomerular basement membrane (GBM) is located between epithelial and endothelial cell layers. **B:** Supramolecular network of type IV collagen is formed by assembly of dimers (NC-NC domain) and tetramers (7S-7S domain). **C:** Three α(IV) chains associate into a triple helical protomer.

membranes but, in contrast to the α5(IV) chain, are absent from the epidermal basement membrane (Kleppel et al., 1989a; Sanes et al., 1990; Yoshioka et al., 1994). The α5(IV) and α6(IV) chains do not always colocalize, and the α6(IV) chain is absent from 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 membrane-bound receptors adhesion, differentiation, cell signaling, and tissue regeneration (Kühn, 1994). The N- and C-terminal region of the α3(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).

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-

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 α1-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 α1 class and 3 for α2. 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 α1 and α2(IV) chains are detected. Next, the α1–α5(IV) chains are present and finally α3-α5(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).

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 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 post-transplant 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 antibodies directed against type IV α3 and α5 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

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.

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 usually 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.

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 pulse-field 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).

COL4A5 MUTATIONS

Large COL4A5 Gene Rearrangements

Thirty-eight large- and medium-size deletions 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′ 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

TABLE 1. Major COL4A5 Gene Rearrangements in Alport Patients[a]

No.	COL4A5	Deletion	Predicted effect on COL4A5 protein	Phenotype: ESRD (age of renal failure)	Deafness	GBM	Family history	Remarks	References
1	Exons 1–51	del 470 kb	No COL4A5 protein	Juvenile	+	ND	+	Renal transplantation, anti-GMB nephritis, del exons 1–3 COL4A6	Antignac et al., 1994; Heidet et al., 1995
2	Exons 1–51	del exons 1–51	No COL4A5 protein	Juvenile (21)	+	ND	De novo mutation	Renal transplant, anti-GMB nephritis	Netzer et al., 1992
3	Exons 1–32	del 210 kb[b]	No COL4A5 protein	ND	ND	+	De novo mutation	Diffuse leiomyomatosis, del exons 1–2 COL4A6, congenital cataract	Heidet et al., 1995
4	Exons 1–30	del 330 kb[b]	No COL4A5 protein	Juvenile (16)	+	+	+	del exons 1–3 COL4A6	Antignac et al., 1994; Heidet et al., 1994
5	Exons 1–18	del 210 kb[b]	No COL4A5 protein	ND (>4)	–	ND	De novo mutation	Bilateral cataract, del exons 1–2 COL4A6, diffuse esophageal leiomyomas	Antignac et al., 1992; Zhou et al., 1993a; Antignac et al., 1994; Heidet et al., 1995
6	Exon 1	del exons 1–4[b] (del 75 kb)	No COL4A5 protein	ND	ND	ND	+	Diffuse leiomyomatosis, del exons 1–2 COL4A6 congenital cataract	Heidet et al., 1995 Dahan et al., 1995
7	Exon 1	del exon 1[b] (del 90 kb)	No COL4A5 protein	ND	ND	+	+	Diffuse leiomyomatosis, del exons 1–2 COL4A6 female patient	Heidet et al., 1995 Dahan et al., 1995
8	Exon 1	Del exons 1–32[b] (del 290 kb)	No COL4A5 protein	ND	ND	ND	ND	Diffuse leiomymatosis, del exons 1–2 COL4A6, congenital cataract, female patient	Heidet et al., 1995
9	Exon 1	del <10 kb[b]	No COL4A5 protein	Juvenile (9)	–	+	De novo mutation	Bilateral cataract, renal transplantation, diffuse esophageal leiomyomatosis, del exons 1–2 COL4A6	Antignac et al., 1992; Zhou et al., 1993a; Antignac et al., 1994; Heidet et al., 1995
10	Exon 1	del 120 kb[b]	No COL4A5 protein	ND	+	+	+	Cataract, diffuse esophageal leiomyomas, del exons 1–2 COL4A6	Antignac et al., 1992; Zhou et al., 1993a; Antignac et al., 1994; Heidet et al., 1995
11	Exon 1	del 15–19 kb	No COL4A5 protein	Juvenile (16)	+	+	ND	Diffuse esophageal leiomyomatosis, del exons 1–2 COL4A6 renal transplantation, cataract	Renieri et al., 1994c; Renieri et al., 1995
12	Exon 1	del 420 kb	No COL4A5 protein	ND	+	ND	De novo mutation	del exons 1–4 COL4A6	Antignac et al., 1994; Heidet et al., 1995
13	Exon 1	del exon 1	No COL4A5 protein	Juvenile (23)	+	+	+	del exons 1–2 COL4A6, cataract	Zhou et al., 1993a

(*continued*)

TABLE 1. Major COL4A5 Gene Rearrangements in Alport Patients[a] (*continued*)

No.	COL4A5	Deletion	Predicted effect on COL4A5 protein	Phenotype			Family history	Remarks	References
				ESRD (age of renal failure)	Deafness	GBM			
14	Exon 1	ND	No COL4A5 protein	Juvenile (25)	+	+	+	Diffuse leiomyomatosis, cataract, del exons 1–2 COL4A6	Dahan et al., 1995
15	Exon 1	ND	No COL4A5 protein	ND	–	+	De novo mutation	Diffuse leiomyomatosis, female patient	Dahan et al., 1995
16	Exon 1	ND	No COL4A5 protein	ND (>16)	+	+	+	Diffuse leiomyomatosis, del exons 1–2 COL4A6	Dahan et al., 1995
17	Exon 1	ND	No COL4A5 protein	–	+	ND	+	Diffuse leiomyomatosis, female patient	Dahan et al., 1995
18	Exons 2–36	del exons 2–36	Truncated COL4A5 (frameshift)	Juvenile (25)	+	ND	+		Renieri et al., 1995
19	Exons 2–19	del exons 2–19	Truncated COL4A5 (frameshift)	ND (>15)	+	+	+		Renieri et al., 1995
20	Exons 4/5	del 4/5–26[b]	Truncated COL4A5	Juvenile	+	+	+		Antignac et al., 1994
21	Exons 4–47	del exons 4–47	No COL4A5 protein,	Juvenile (<20)	+	+	ND	Renal transplantation, anti-GBM nephritis in two related patients	Ding et al., 1994
22	Exons 4–13	del exons 4–13	Truncated COL4A5 (inframe)	Juvenile (27)	ND	ND	+	(no COL4A3 protein in GMB)	Renieri et al., 1995
23	Exons 14–51	del exons 14–51	Truncated COL4A5	Juvenile (<20)	+	+	+	Renal transplantation, anti-GBM nephritis	Ding et al., 1994
24	Exon 17	del exon 17	Truncated COL4A5, del 18 amino acids (inframe)	Juvenile (average 24)	+	+	+		Renieri et al., 1995
25	Exons 19–22	del exons 19–22	Truncated COL4A5	Juvenile (average 23)	+	ND	+	Renal transplantation	Renieri et al., 1995
26	Exons 20–26	del exons 20–26[b]	Truncated COL4A5	ND	+	+	+		Antignac et al., 1994
27	Exons 20–21	del exons 20–21	Truncated COL4A5 (inframe)	Juvenile (18)	+	ND	ND		Renieri et al., 1995
28	Exon 22	del exons 22–28; del exons 38–51	truncated COL4A5	Juvenile (17)	+	+	+	Renal transplantation anti-GBM nephritis	Smeets et al., 1992
29	Exons 28–32	dup exons 28–32	Aberrant COL4A5	Juvenile (23)	+	+	+		Renieri et al., 1995
30	Exons 34–51	del exons 34–51 (del 300 kb)	Truncated COL4A5	ND	ND	ND	ND		Flinter, 1993
31	Exon 38	del exons 38–51 (34 kb deletion)	Truncated COL4A5	Juvenile (26)	+	ND	+		Netzer et al., 1992
32	Exons 38–46	Ins exons 38–46	Truncated COL4A5	ND	ND	ND	ND		Flinter, 1993
33	Exons 38–51	dup 35 kb of 14 exons at 3´ end	Aberrant COL4A5	Adult (33)	+	+	De novo mutation	Renal transplantation	Boye et al., 1991; Vetrie et al., 1992

(*contined*)

TABLE 1. Major COL4A5 Gene Rearrangements in Alport Patients[a] (*continued*)

No.	COL4A5	Deletion	Predicted effect on COL4A5 protein	Phenotype: 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	+	ND	+		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)	+	+	+	Renal transplantation	Boye et al., 1991; Vetrie et al., 1992
36	Ins/del 46–47	ins 10–15 kb exon 46/47, (del 25 kb)	Aberrant COL4A5	ND (>16)	+	+	+		Boye et al., 1991; Vetrie et al., 1992
37	Exons 49–51	del 10 kb at 3′ end	Truncated COL4A5	Juvenile (15)	+	+	+		Saito et al., 1994
38	Exon 40	del exon 40	Truncated COL4A5 (inframe)	ND (>13)	+	ND	+	Renal transplantation	Renieri et al., 1995

[a]Patients are numbered with respect to the location of the mutation. Mutations are designated according to Beaudet and Tsui (1993).
[b]Precise localization 5′ and 3′ breakpoints of deletions has not been determined.
ESRD, end-stage renal disease (age at renal failure or age at time report, in years); GBM, glomerular basement membrane; GBM+, electron microscopy of GBM shows structural abnormalities diagnostic for Alport syndrome; del, deletion; ins, insertion; dup, duplication; ND, no data available.

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 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 hematuria, 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 Lys664Asn 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 different 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 = 177 families) (Renieri et al., 1995), German (n = 20 families) (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

TABLE 2. Small Mutations of the COL4A5 Gene in Alport Patients[a]

No.	COL4A5	Mutation	Effect on coding sequence[b]	Phenotype[c]			Family history	Remarks	References
				ESRD (age of renal failure)	Deafness	GBM			
1	Exon 1	203A→G	Met1Val	Juvenile (28)	ND	ND	+	Other start codon, shorter COL4A5 protein	Renieri et al., 1996
2	Exon 3	353insA/358insT	Frameshift Gly51/Phe52	Juvenile (18)	+	ND	+	Stop 101/102 codons downstream	Heiskari et al., 1996
3	Exon 3	363G→A	Gly54Asp	Adult (40)	+	ND	+	Renal transplantation, interruption Gly-X-Y	Turco et al., 1995
4	Exon 7	588G→A	Gly129Glu	Juvenile (30)	+	+	+	Interruption Gly-X-Y, possible splice site mutation	This article
5	Exon 7	588G→T	Gly129Val	Juvenile (29)	+	+	+	Interruption Gly-X-Y, possible splice site mutation	Heiskari et al., 1996
6	Exon 9	722G→C	Gly174Arg	ND	ND	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
7	Exon 9	731G→C	Gly177Arg	Adult (34)	+	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
8	Exon 11	835/836delC	Frameshift Pro212	Juvenile (21)	+	ND	+	Renal transplantation, stop 8 codons downstream	Renieri et al,. 1996
9	Intron 11	848-3C→A	Acceptor splice site	ND (>17)	–	+	+	mRNA; exon 12 spliced out	Boye et al., 1995
10	Exon 12	848G→A	Gly216Arg	Juvenile (15)	+	+	+	Interruption Gly-X-Y, possible splice site mutation, renal transplantation	Heiskari et al., 1996
11	Exon 12	857G→A	Gly219Ser	ND (>18)	–	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
12	Intron 12	889+ 1G→A	Donor splice site	Juvenile (30)	+	+	+	Renal transplantation	Heiskari et al., 1996
13	Exon 13	960/962del2	Frameshift Glu254	Juvenile (15)	+	ND	+	Stop 11 codons downstream, renal transplantation	Heiskari et al., 1996
14	Exon 13	960/962del2	Frameshift Glu254	Juvenile	–	+	De novo mutation		Kawai et al., 1996
15	Intron 13	983-1del7	Acceptor splice site	Juvenile	ND	+	+		Naito et al., 1996
16	Intron 13	983-1del7	Acceptor splice site	Adult	–	+	+		Kawai et al., 1996
17	Exon 15	1061G→T	Glu287Stop	Juvenile (<30)	+	ND	+	Renal transplantation	This article
18	Exon 15	1061G→T	Glu287Stop	Juvenile (17)	+	ND	+		Heiskari et al., 1996
19	Exon 15	1068G→T	Gly289Val	Juvenile (30)	–	+	+	Renal transplantation female patient, skewed X-inactivation, interruption Gly-X-Y, second COL4A5 mutation on same allele (see no.110)	Guo et al., 1995
20	Exon 15	1077G→T	Gly292Val	Juvenile (15)	+	ND	+	Interruption Gly-X-Y	Heiskari et al., 1996

(*continued*)

TABLE 2. Small Mutations of the COL4A5 Gene in Alport Patients[a] (*continued*)

No.	COL4A5	Mutation	Effect on coding sequence[b]	Phenotype[c]			Family history	Remarks	References
				ESRD (age of renal failure)	Deafness	GBM			
21	Exon 15	1086delC	Frameshift Arg297	Juvenile (4)	+	+	+	Stop 49 codons downstream, renal transplantation	Heiskari et al., 1996
22	Intron 15	1094-2A→G	Acceptor splice site	Adult (39)	+	ND	+	Renal transplantation	Heiskari et al., 1996
23	Intron 15	1094-1G→C	Acceptor splice site	Juvenile (28)	+	+	+		Heiskari et al., 1996
24	Exon 17	1147insT	Frameshift Gly316	Juvenile (27)	+	ND	De novo mutation	Stop 1 codon downstream, renal transplantation	Heiskari et al., 1996
25	Exon 17	1175G→A	Gly325Arg	Juvenile/adult (average 40)	+	+	+	Interruption Gly-X-Y	Knebelmann et al., 1992
26	Exon 17	1175G→T	Gly325Stop	ND	–	+	+		Kawai et al., 1996
27	Exon 17	1175G→A	Gly325Arg	Adult (>36)	+	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
28	Exon 17	1175G→A	Gly325Arg	ND	–	+	+	Interruption Gly-X-Y	Heiskari et al., 1996
29	Exon 17	1176G→A	Gly325Glu	ND (>9)	–	+	De novo mutation	Interruption Gly-X-Y	Renieri et al., 1992a; Massella et al., 1994
30	Intron 18	1234del4	Donor splice site	Juvenile (26)	+	ND	ND		Heiskari et al., 1996
31	Intron 18	1234del4	Donor splice site	Juvenile (19)	+	+	ND		Heiskari et al., 1996
32	Exon 19	1271–1275delA	Frameshift Gly359	ND	ND	+	+		Kawai et al., 1996
33	Exon 19	1294del9	Inframe deletion Gly365-Pro367	Juvenile (8)	–	+	+	Shorter COL4A5 protein	Heiskari et al., 1996
34	Exon 19	1296G→A	Gly365Glu	Juvenile (26)	+	ND	+	Interruption Gly-X-Y, renal transplantation	Heiskari et al., 1996
35	Exon 19	1314G→A	Gly371Glu	Juvenile (19)	+	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
36	Exon 19	1319C→T	Arg373Stop	Juvenile (18)	+	+	+		Heiskari et al., 1996
37	Exon 19	1323G→C	Gly374Ala	ND (>9)	–	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
38	Exon 19	1350G→A	Gly383Asp	Juvenile	+	+	+	Interruption Gly-X-Y	Kawai et al., 1996
39	Intron 19	1367 + 1G→A	Donor splice site	ND (>19)	+	ND	+		Renieri et al., 1996
40	Intron 19	1367 + 2T→G	Donor splice site	Juvenile (13)	+	ND	ND		Renieri et al., 1996
41	Exon 20	1410G→A	Gly400Glu	Adult (35)	+	ND	ND	Interruption Gly-X-Y	Boye et al., 1995
42	Exon 20	1419G→T	Gly406Val	ND (>23)	+	+	De novo mutation	Interruption Gly-X-Y	Boye et al., 1995
43	Exon 20	1419G→T	Gly406Val	Adult (average 31)	+	ND	ND	Interruption Gly-X-Y	Renieri et al., 1996
44	Exon 20	1419insGGGG	Frameshift Gln407	ND(>19)	+	ND	+	Stop 4 codons downstream	Renieri et al., 1996
45	Exon 20	1482insA	Frameshift Pro428	Juvenile (23)	+	ND	+	Stop 19 codons downstream	Renieri et al., 1996
46	Exon 20	1428G→A	Gly409Asp	ND (>16)	+	ND	+	Interruption Gly-X-Y	Renieri et al.,1996
47	Exon 20	1533T→G	Ile444Ser	Juvenile	+	+	+	Female patient	Kawai et al., 1996
48	Exon 20	1533T→G	Ile444Ser	Juvenile	–	+	+		Kawai et al., 1996
49	Exon 20	1533T→G	Ile444Ser	ND	–	+	De novo mutation	Female patient	Kawai et al., 1996
50	Intron 20	1542-2A→G	Acceptor splice site	ND (>23)	+	ND	+		This article
51	Exon 21	1549delAT	Frameshift Ile450	Juvenile (13)	+	ND	+	Multiple renal transplant rejection, stop 1 codon downstream	Boye et al., 1995

(*continued*)

TABLE 2. Small Mutations of the COL4A5 Gene in Alport Patients[a] (*continued*)

No.	COL4A5	Mutation	Effect on coding sequence[b]	Phenotype[c] ESRD (age of renal failure)	Deafness	GBM	Family history	Remarks	References
52	Exon 21	1567del9	Inframe deletion Pro-Gly-Pro	ND	ND	ND	De novo mutation	Shorter COL4A5 protein	Renieri et al., 1996
53	Exon 21	1599G→A	Gly466Glu	ND	+	+	+	Interruption Gly-X-Y	Kawai et al., 1996
54	Exon 22	1683G→A	Gly494Asp	ND (>20)	+	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
55	Exon 22	1685del36	Inframe deletion	Juvenile (average 22)	+	ND	ND	Shorter COL4A protein	Renieri et al., 1996
56	Exon 22	1685del36	Inframe deletion	Juvenile (27)	+	ND	+	Shorter COL4A5 protein	Renieri et al., 1996
57	Exon 23	1763G→T	Gly521Cys	Juvenile (29)	+	ND	+	Renal transplantation	Zhou et al., 1992b
58	Exon 23	1767/1768delA	Frameshift Thr 523	ND	ND	ND	+	Stop 33 codons downstream	Renieri et al., 1996
59	Exon 24	1902G→C	Gly567Ala	Juvenile (16)	+	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
60	Intron 24	1981 + 1G→T	Donor splice site	ND	ND	ND	ND		Renieri et al., 1996
61	Exon 25	2028G→T	Gly609Val	Juvenile (23)	+	ND	ND	Renal transplantation; interruption Gly-X-Y	This article
62	Exon 25	2115G→T	Gly638Val	ND(>29)	+	+	+	Interruption Gly-X-Y	Boye et al, 1995
63	Exon 25	2115G→C	Gly638Ala	ND (>27)	–	+	+	Interruption Gly-X-Y	Boye et al., 1995
64	Exon 26	2159G→A	Gly653Arg	Juvenile (19)	+	+	+	Interruption Gly-X-Y; renal transplant rejection	Boye et al., 1995
65	Exon 26	2219delG	Frameshift Arg673	ND	ND	ND	De novo mutation	Stop 4 codons downstream	Renieri et al., 1996
66	Exon 27	2253G→T	Gly684Val	Adult	+	+	+	Interruption Gly-X-Y, female patient	Kawai et al., 1996
67	Exon 27	2348G→C	Donor splice site/ Gly716Arg	Juvenile	+	+	+	Interruption Gly-X-Y or aberrant COL4A5 mRNA	Kawai et al., 1996
68	Exon 28	2421G→A	Gly740Glu	Juvenile	ND	+	ND	Interruption Gly-X-Y	Kawai et al., 1996
69	Exon 29	2517G→A	Gly772Asp	Juvenile	–	+	+	Interruption Gly-X-Y	Kawai et al., 1996
70	Exon 29	2588G→A	Gly796Arg	ND	+	+	+	Interruption Gly-X-Y	Boye et al., 1995
71	Intron 29	2597 +2delT	Donor splice site	Juvenile (17)	+	ND	+		Renieri et al., 1996
72	Exon 31	2756G→A	Gly852Arg	ND	–	+	+	Interruption Gly-X-Y	Kawai et al., 1996
73	Exon 31	2799G→A	Gly866Glu	Adult (>31)	+	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
74	Exon 31	2799G→A	Gly866Glu	ND (>7)	–	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
75	Exon 31	2807G→A	Gly869Arg	ND (>24)	+	+	+	Interruption Gly-X-Y	Boye et al., 1995
76	Exon 31	2807G→A	Gly869Arg	Juvenile (10)	–	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
77	Exon 31	2807G→A	Gly869Arg	ND (>13)	+	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
78	Exon 31	2816G→C	Gly872Arg	ND	+	ND	+	Interruption Gly-X-Y	Boye et al, 1995
79	Exon 31	2825 del A	Frameshift Ser897	Juvenile (12)	+	+	+	Multiple renal transplant rejection, stop 21 codons downstream	Boye et al., 1995
80	Exon 33	3004insT	Frameshift Gly935	ND	ND	ND	ND	Stop 7 codons downstream	Hertz et al., in preparation
81	Exon 33	3025del3	Inframe deletion Ser942	ND	ND	ND	De novo mutation	Interruption Gly-X-Y, shorter COL4A5	Renieri et al., 1996

(*continued*)

TABLE 2. Small Mutations of the COL4A5 Gene in Alport Patients[a] (*continued*)

No.	COL4A5	Mutation	Effect on coding sequence[b]	Phenotype[c]				Remarks	References
				ESRD (age of renal failure)	Deafness	GBM	Family history		
82	Exon 34	3141-3144delA	Framshift Gly982	Juvenile (average 16)	+	ND	+	Renal transplantaton, stop 13 codons downstream	Peissel et al., 1994
83	Exon 34	3166delG	Frameshift Asp989	ND (>9)	+	+	+	Stop 6 codons downstream	This article
84	Exon 36	3414C→G	Ser1071Stop	ND	ND	ND	ND	Stop at codon 1071	Hertz et al., in preparation
85	Exon 37	3513G→T	Gly1104Val	ND	–	+	+	Interruption Gly-X-Y	Kawai et al., 1996
86	Exon 37	3532-3533delA	Frameshift Thr1111	ND	ND	ND	+	Stop 40 codons downstream	Renieri et al., 1996
87	Exon 37	3539insCCTG	Frameshift Gly1113	ND (>3)	–	ND	De novo mutation	Stop 24 codons downstream	Renieri et al., 1996
88	Exon 38	3629G→A	Gly1143Ser	Adult (average 39)	+	+	+	Interruption Gly-X-Y	Renieri et al., 1994b
89	Exon 38	3630G→A	Gly1143Asp	Juvenile (25)	–	+	+	Interruption Gly-X-Y, renal transplantation rejection	Zhou et al., 1992a
90	Intron 38	3656 + 1G→C	Donor splice site	Juvenile (18)	+	+	+	Renal transplantation, anti-GBM nephritis, mRNA: exon 38 spliced out	Netzer et al., 1993
91	Exon 39	3709/3710delG	Frameshift Gly1170	ND(>19)	+	+	+	Stop 128 codons downstream	Renieri et al., 1994a
92	Exon 39	3744del7	Frameshift Lys1181	Juvenile (19)	+	ND	+	Renal transplantation, stop 115 codons downstream	Renieri et al., 1993
93	Exon 39	3746G→C	Gly1182Arg	Juvenile	+	+	+	Interruption Gly-X-Y	Kawai et al, 1996
94	Exon 41	3813/3814/3815delG	Frameshift Gly1205	Juvenile (16)	+	ND	+	Stop 93 codons downstream	Lemmink et al., 1994a
95	Exon 41	3819del4	Frameshift Asp1206	Juvenile (average 22)	+	+	+	Renal transplantation, stop 91 codons downstream	Renieri et al., 1993
96	Exon 41	3912del52	Frameshift Pro1240	ND	–	+	+	Stop 41 codons downstream	Kawai et al., 1996
97	Exon 41	3923G→T	Gly1241Cys	ND (>23)	–	+	De novo mutation	Interruption Gly-X-Y	Boye et al., 1995
98	Exon 41	3959ins4	Frameshift Arg1253	ND	+	+	+		Kawai et al., 1996
99	Intron 41	3993-1G→A	Acceptor splice site	Juvenile (16)	+	+	+	mRNA: exon 42 spliced out, stop at nucleotide 4129, and G deletion at 3993 introducing frame-shift with stop 16 codons downstream	Lemmink et al., 1994a; Lemmink et al., 1993

(*continued*)

TABLE 2. Small Mutations of the COL4A5 Gene in Alport Patients[a] (*continued*)

No.	COL4A5	Mutation	Effect on coding sequence[b]	Phenotype[c]			Family history	Remarks	References
				ESRD (age of renal failure)	Deafness	GBM			
100	Exon 42	4010G→A	Gly1270Ser	ND	ND	ND	ND	Interruption Gly-X-Y	Hertz et al., in preparation
101	Exon 42	4015/4016delC	Frameshift Pro1272	Juvenile (20)	+	ND	+	Renal transplant rejection, stop 26 codons downstream	Renieri et al., 1994a
102	Exon 42[d]	4058del7	Frameshift Asn1286	Juvenile (13)	+	+	+	Renal transplantation, stop 10 codons downstream	Guo et al, 1993
103	Exon 43	4192/4193delT	Frameshift Phe 1331	ND(>8)	ND	ND	+	Stop 3 codons downstream	Renieri et al., 1996
104	Intron 43	4200-2A→G	Acceptor splice site	Juvenile (30)	ND	ND	ND	mRNA: exon 44 spliced out	Renieri et al., 1996
105	Exon 44	4245del6/ 4241G→A	Inframe deletion Pro-Gly/Gly1348Glu	ND	+	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
106	Exon 45	4338G→T	Gly1379Val	ND	ND	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
107	Exon 45	4363insTCCT	Frameshift Gly1388	ND	ND	ND	De novo mutation	Female patient, stop 30 codons downstream	Massella et al., 1994
108	Exon 46	4430C→T	Arg1410Cys	Juvenile (average 26)	+	ND	+	Additional Cys residue	Renieri et al., 1996
109	Exon 46	4463G→T	Gly1421Trp	Adult (>33)	–	ND	+	Interruption Gly-X-Y	Renieri et al., 1996
110	Exon 46	4467C→T	Arg1422Cys	Juvenile (30)	–	+	+	Female patient, skewed X-inactivation, other COL4A5 mutation (see no. 19)	Guo et al., 1995
111	Exon 47	4517dup36	Duplication 12 codons from Gly1439	ND	ND	ND	+		Hamalainen et al., 1996
112	Exon 47	4553G→A	Gly1451Ser	ND (>8)	–	+	ND		This article
113	Exon 47	4637del2	Frameshift Gly1479	Juvenile (20)	+	ND	+	Renal transplantation, stop at codon 1485	Hertz et al., 1995
114	Exon 47	4546insT	Frameshift 1449Pro	Juvenile (12)	+	ND	De novo mutation	Renal transplantation, stop 36 codons downstream	Renieri et al., 1996
115	Exon 47	4686C→A	Ala1498Asp	Adult (37)	–	ND	+		Tverskaya et al, 1996
116	Intron 47	4712+1G→C	Donor splice site	Juvenile (18)	+	+	+	mRNA; exon 47 spliced out, inframe del 71 amino acids	Nakazato et al., 1995
117	Intron 47	4712+1G→C	Donor splice site	Juvenile	–	+	+		Kawai et al., 1996
118	Exon 48	4749insT	Frameshift Met1516	Juvenile (18)	+	+	+	Stop 9 codons downstream	Nakazato et al., 1993
119	Exon 48	4751C→A	Pro1517Thr	Juvenile[e] (average 16)	+	ND	+		Lemmink et al., 1993
120	Exon 48	4751C→A	Pro1517Thr	ND (>15)	–	ND	ND		Renieri et al., 1996

(*continued*)

TABLE 2. Small Mutations of the COL4A5 Gene in Alport Patients[a] (*continued*)

No.	COL4A5	Mutation	Effect on coding sequence[b]	Phenotype[c]			Family history	Remarks	References
				ESRD (age of renal failure)	Deafness	GBM			
121	Exon 48	4815G→C	Trp1538Ser	Adult (33)	–	+	+	Renal transplantation	Smeets et al., 1992
122	Exon 48	4889C→T	Arg1563Stop	Juvenile (23)	+	ND	+	Renal transplantation	Zhou et al., 1993b
123	Exon 48	4889C→T	Arg1563Stop	Juvenile (16)	+	ND	+		This article
124	Exon 48	4890G→A	Donor splice site	Juvenile (29)	+	ND	+	Renal transplantation	Zhou et al., 1993b
125	Exon 48	4890G→A	Donor splice site	ND (>21)	+	+	+		Zhou et al., 1993b
126	Exon 48	4890G→A	Donor splice site	Juvenile (average 25)	+	+	+	Renal transplantation, mRNA; exon 48 spliced out, frameshift with stop 10 codons downstream	Lemmink et al., 1993; Lemmink et al., 1994a
127	Exon 49	4891-3del32	Acceptor splice site	Adult	–	+	De novo mutation	Female patient	Kawai et al., 1996
128	Exon 49	4893G→C	Cys1564Ser	Adult (33)	+	+	+		Zhou et al., 1991b
129	Exon 49	4989G→A	Gly1596Asp	ND(>19)	+	ND	+		Renieri et al., 1996
130	Exon 49	4991/4992insA	Frameshift 1597Tyr	ND (>12)	+	+	+	Stop at codon 1597	Nakazato et al., 1994
131	Exon 49	5005G→C	Donor splice site Met 1601 Ile	Juvenile (15)	–	+	+	mRNA; multiple transcripts with premature stops	Nomura et al., 1993 Kawai et al., 1996
132	Exon 49	5005+1Gins10	Donor splice site Frameshift Met 1601	Adult (>30)	+	+	+	mRNA; ins 9 bp exon 49, stop 110 nucleotides downstream	Lemmink et al., 1993 Lemmink et al., 1994a
133	Exon 50	5032/5033delG	Frameshift Gly1611	Juvenile (12)	+	+	+	Renal transplantation stop 3 codons downstream	Renieri et al., 1993
134	Exon 50	5148T→G	Leu1649Arg	Adult (>31)	+	+	+		Barker et al., 1996
135	Exon 50	5170delC	Frameshift Asp1656	ND	–	+	De novo mutation	Female patient, stop 10 codons downstream	Kitagawa et al., 1995
136	Intron 50	5179-2A→G	Acceptor splice site	Juvenile (11)	+	+	+	mRNA; cryptic splice site, 5178del10, stop 4 codon downstream	Nakazato et al., 1995
137	Exon 51	5191del8/ 5195 del8	Frameshift 1665Thr	ND	ND	ND	ND	Stop 8 codons downstream	Boye et al., 1993
138	Exon 51	5237C→T	Glu1679Stop	ND (>15)	–	+	+	Putative germline mutation in mother	Nakazato et al., 1994

[a]Patients are numbered with respect to the location of the mutation. Mutations are designated according to Beaudet and Tsui (1993).
[b]Amino acid residue numbering for human α5(IV) chain is based on Zhou et al., 1994a.
[c]Other clinical features of Alport syndrome, such as perimacular flecks; anterior lenticonus are not included because insufficient data were available.
[d]Additional mutations further downstream: 4068A→T, and 4071C→T (Guo et al., 1993).
[e]Average age of renal failure based on data from two deceased brothers.
ESRD, end-stage renal disease (age at renal failure or age at time report, in years); GBM, glomerular basement membrane; del, deletion, ins, insertion GBM+, electron microscopy of GMB shows structural abnormalities diagnostic for Alport syndrome; dup, duplication; ND, no data available.

TABLE 3. COL4A3 and COL4A4 Gene Mutations[a]

No.	COL4A3	Mutation[b]	Effect on coding sequence[c]	Phenotype: ESRD (age of renal failure)	Phenotype: Deafness	Phenotype: GBM	Remarks	References
1	Exon 5	4441C→T homozygous	Arg1481Stop	Juvenile (11)	+	+	Renal transplantation, consanguinous parents	Mochizuki et al., 1994
2	Exons 4;5	4559C→G; 4441C→T compound heterozygote	Ser1524Stop; Arg1481Stop	ND	ND	ND	Renal transplantation, anti-GBM *nephritis*	Lemmink et al., 1994b
3	Exon 5	4414del5/ 4419del5 homozygous	Frameshift Leu1474	Juvenile (9)	+	+	Stop 33 codons downstream, renal transplantation; anti-GBM nephritis female, (male and female patient)	Mochizuki et al., 1994
4	Exon 5	4414del5/ 4419del5 heterozygous	Frameshift Leu1474	ND(>5)	ND	+	Stop 33 codons downstream, female patient	Lemmink et al., 1994b
5	Intron 1	G→T	Frameshift Arg1643	Juvenile (14)	+	ND	2 aberrant mRNAs: insertion part Alu repeat, possibly consanguinous	Knebelmann et al., 1995
6	Exon 6	4346del7 homozygous	Frameshift 1449Thr	Juvenile (20)	+	+	Renal transplantation; anti-GBM *nephritis*, stop 76 codons downstream, female patient	Ding et al., 1995
No.	COL4A4							
1	Collagenous domain	2298G→A heterozygous	Gly897Glu	None	--	Thin	Family with autosomal dominant familial benign hematuria	Lemmink et al., 1996
2	Collagenous domain	3809G→A homozygous	Gly1201Ser	Juvenile (14)	–	+	Consanguinous parents, female patient	Mochizuki et al., 1994
3	Collagenous domain	3921C→A homozygous	Ser1238Stop	Juvenile (18)	–	ND	Renal transplantation, female patient	Mochizuki et al., 1994

[a]Patients are numbered with respect to the location of the mutation. Mutations are designated according to Beaudet and Tsui (1993).
[b]Exon numbering starts at 3′ end.
[c]Nucleotide and amino acid residues are numbered according to Mariyama et al. (1994) for COL4A4. GBM+, electron microscopy of GBM shows structural abnormalities diagnostic for Alport syndrome.
ESRD, end-stage renal disease; GBM, glomerular basement membrane; del, deletion, ins, insertion; dup, duplication; ND, no data available.

(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 X-linked 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.

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 (4889CαT) 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 (4890GαA) 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 4890GαA 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.

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 effect 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.

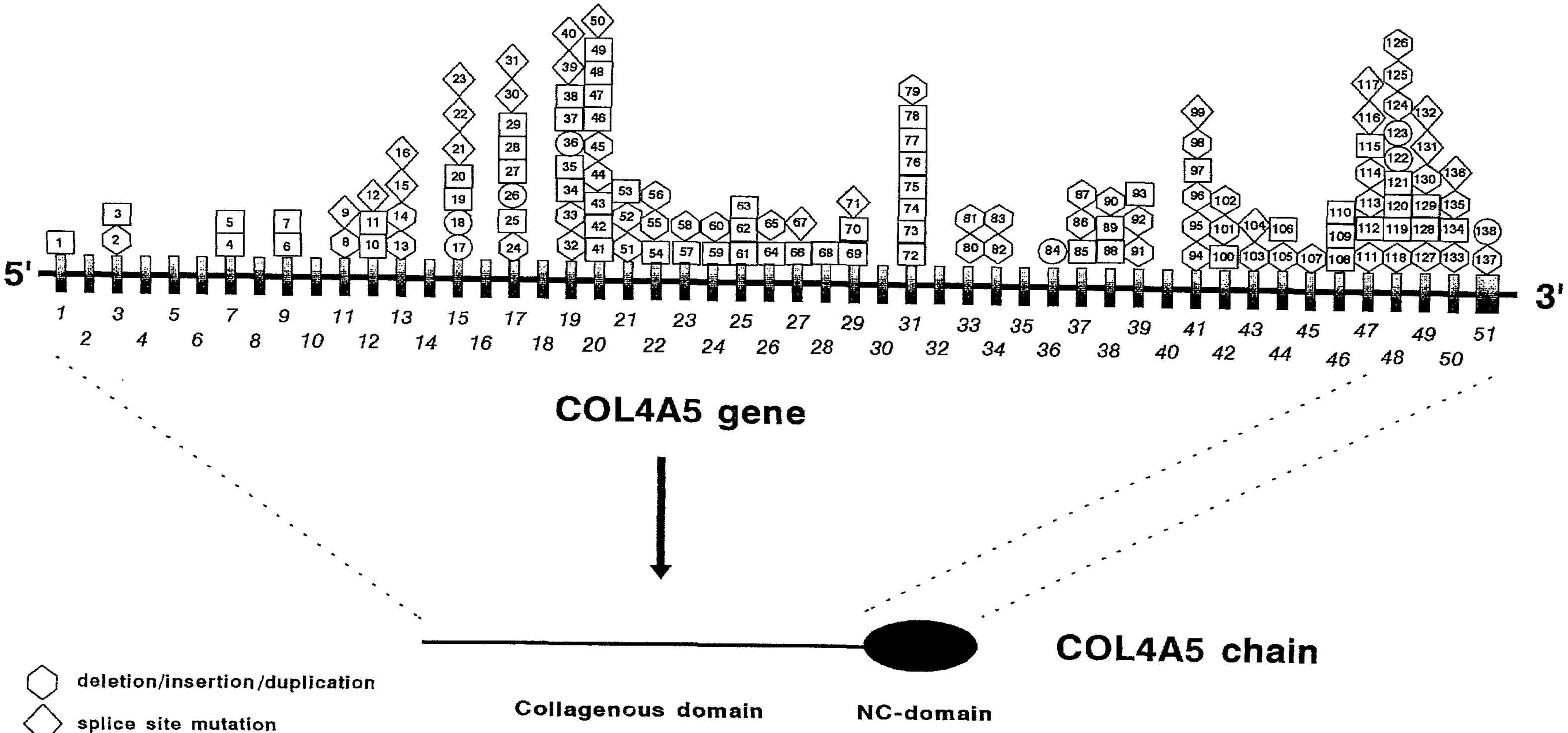

FIGURE 2. Localization and distribution of small mutations in the collagen α5(IV) chain. Boxed numbers, 1–138, correspond to the COL4A5 gene mutations described in Table 2. Numbers in italics, 1–51, indicate individual COL4A5 exons.

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).

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.

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 kindred 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.

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).

The absence of mutant α(IV) chains can distort association with the other normal α(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 α3 and α5 epitopes, although not always at the mutated chain. A COL4A5 muta-

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 α5(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).

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, 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).

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