View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Kidney International, Vol. 44 (1993), pp. 1316–1321

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

CAIYING GUO, BOUDEWIJN VAN DAMME, RITA VAN DAMME-LOMBAERTS, HERMAN VAN DEN BERGHE, JEAN-JACQUES CASSIMAN, and PETER MARYNEN

Center for Human Genetics, University of Leuven, and Departments of Pathology and Pediatrics, University Hospital, Leuven, Belgium

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. PCR conditions were optimized to amplify the COL4A5 cDNA from lymphoblasts and kidney tissue. Sequencing of the COL4A5 mRNA isolated from the kidney of an Alport syndrome patient revealed two differences with the published sequence. One divergence, the insertion of an 18 bp sequence between exon 11 and 10 of the COL4A5 mRNA added two Gly-X-Y triplets to the COL4A5 sequence and was subsequently found in the mRNA of four normal kidney mRNA samples. This sequence was absent in all white blood cell RNA samples sequenced by us, indicating tissue specific splicing with the presence of an additional exon in kidney COL4A5 mRNA. This finding of differential splicing of COL4A5 mRNA in kidney and white blood cells might affect the use of white blood cell mRNA for the analysis of Alport mutations. Second, a complex mutation was detected in the mRNA from the AS patient introducing a premature stop codon in the message, deleting part of the triple helical domain and the complete NC domain. The mother of the patient was shown to be heterozygous for this mutation.

Alport syndrome (AS) is an X-linked dominant disease characterized mainly by a progressive nephritis with hematuria. The clinical picture is heterogenous with a variable age of end-stage renal disease, and a variable association with ocular lesions and deafness. At the ultrastructural level the disease is characterized by the patchy splitting of the lamina densa of glomerular basement membrane. A lack of fixation of antibodies to collagen type 4 chains (α3-5) is also sometimes observed.

Genetic analysis maps the AS locus to chromosome Xq22 [1–3]. This region carries the COL4A5 gene [4] and subsequent analysis detected different point mutations as well as larger deletions in the COL4A5 gene of AS patients [5–11]. Linkage analysis did not show evidence for heterogeneity at the locus level, although the presence of a second AS gene close to the COL4A5 gene could not be excluded [12]. Analysis of the different COL4A5 alleles might, however, permit a correlation of the clinical pathology with specific mutations in the COL4A5 gene and investigation of the molecular mechanisms underlying AS pathology.

The COL4A5 gene spans more than 100 kb of the human genome and contains more than 50 exons. Mutation screening strategies based on Southern hybridization or exon sequencing are rather insensitive or very time consuming. As an alternative cDNA sequencing was developed based on the illegitimate transcription of the COL4A5 gene in lymphoblasts [6, 13, 14], since the transcription of the COL4A5 gene appears to be restricted to the kidney [15]. This implicitly requires the illegitimately transcribed mRNA to be a *bona fide* version of the normal tissue specific mRNA, which is normally accepted to be the case.

To investigate this hypothesis and to allow a large scale screening for AS mutations, we optimized conditions to amplify the COL4A5 cDNA in six overlapping fragments from poly(A^+) RNA isolated from white blood cells and from kidney tissue. Comparison of the sequences of the mRNA of both tissues strongly suggests alternative splicing with the presence of an additional exon in the COL4A5 mRNA in kidney, which is absent in white blood cell mRNA. The presence of the additional exon was then confirmed on a second Alport kidney sample, three normal kidney samples and a primary culture of kidney cells. Sequencing of the cDNA from an AS patient showed a complex deletion/missense mutation which was also detected in the DNA of the mother of the patient.

Methods

Patients and samples

The patient was a 2.5-year-old boy with macroscopic hematuria without fever or pain and a proteinuria of 300 mg/day, with a mildly decreased creatinine clearance of 72 ml/min/1.73 m^2. A kidney biopsy at that time showed focal sclerosing lesions. Alterations of the basement membrane, characteristic for Alport's disease, were not seen at electron microscopy of the only glomerulus available. With continued hematuria a second biopsy was taken two years later and this showed minimal mesangial proliferation, with negative immunohistology. A third biopsy was taken at the age of 8, when the child became clinically deaf due to a perception defect. Now interstitial foam

Received for publication May 14, 1993
and in revised form July 22, 1993
Accepted for publication July 26, 1993

© 1993 by the International Society of Nephrology

Table 1. Collagen 4A5 PCR primer list

		Forward primers	Reverse primers
Fragment 1	1st PCR:	ACACCAGGTCTTCCTGGAATG	TGGAGTCCTTTATCACCTGG
	nested:	CAAGGTATTCCCGGATGCAAT	TATCTCATCACTAGGAGGAAT
Fragment 2	1st PCR:	TTTCCTGGAGAAAGGGGTCAG	CTGGTATTCCTGGCTGGCCT
	nested:	TGAAGGACCACCTGGAATTTCC	ATGCCTTTTTCACCTACTGG
Fragment 3	1st PCR:	TTAAGGGTGAAAGAGGTCCC	ACCTGCTTTTCCTGGAAGCCCTG
	nested:	GGAATATAGGGCCTATGGGT	GATCCCTGGACTGCCTCTTT
Fragment 4	1st PCR:	CTGCCAGGAATAGGTGTTCA	ACCACCTACAGGACCAGGTT
	nested:	CAACCTGGTTTACATGGAAT	TTCCCAGGAGGGCCACTAAT
Fragment 5	1st PCR:	CTGGTATCAAAGGTTCTGTG	ATCAAAGCCAGGGAGTCCAT
	nested:	TACCAGGAACCCCTGGAGCA	GGTAAGCCTTGAGGTCCTTG
Fragment 6	1st PCR:	GTCCTTCAGGACAGAGTATC	ATTCGTGTCCTCAAGTCTCCTGC
	nested:	ATGCTGGTCCTCCAGGAATC	CAGCGTTTCTGACTGAGGTT

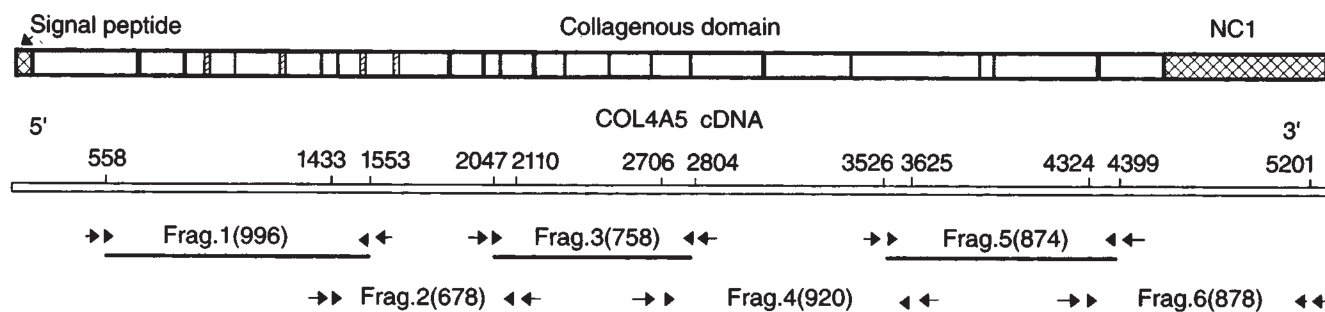


Fig. 1. *Strategy for amplification of the COL4A5 cDNA by PCR.* A scheme of the COL4A5 protein according to [7]. The aminoterminal signal peptide, the carboxyterminal NC1 domain and the collagenous domain are shown. Interruptions of the triple helical domain by non-collagenous sequences are indicated by vertical bars and cross hatched regions. The localization of the six amplified fragments (with their length in bp between brackets) is shown with the localization of the primers (arrows) and the nested primers (arrowheads). The boundaries of the fragments are indicated on the bar representing the COL4A5 cDNA with the numbering according to [7].

cells were found. The material for electron microscopy contained no glomeruli. The renal function deteriorated progressively, and hemodialysis was started at the age of 13. He was transplanted with a cadaver kidney one year later. At that time a nephrectomy was performed, and fragments of this kidney were used for molecular biological investigation. The child died two and a half months later from an Epstein Barr virus-related transplant lymphoma.

A kidney biopsy of his mother at the age of 28, for persistent hematuria and proteinuria after the birth of her son, showed focal sclerosis with the presence of interstitial foam cells. Immunohistochemical examination was negative. Electron microscopy of the specimen showed a thin basement membrane but no lesions characteristic for Alport's disease. However, recutting of the same glomeruli at the time of renal failure of her son revealed the typical basket wave appearance of the basement membrane, in some areas not present in the original sections. From the patient a fragment of the puncture biopsy was available for molecular biological investigation.

Four other kidney samples were obtained. One sample was obtained by nephrectomy at the moment of transplantation of an Alport patient. Two samples were normal tissue obtained by nephrectomy for renal carcinomas, and the fourth sample of normal tissue was obtained by nephrectomy for transitional cell carcinoma of the renal pelvis.

Kidney cells were obtained by a direct culture of normal kidney tissue obtained by nephrectomy for renal cell carcinoma.

White blood cells where obtained by fractionation of peripheral blood with dextran 500. EBV transformed cells of Alport patients and normal controls were established according to standard procedures and maintained in DME:F12 (1:1) containing 4% Ultroser (Gibco).

Reverse transcription PCR

RNA was extracted from cultured cells and from tissues using the acid guanidinium thiocyanate-phenol method [16]. Poly(A^+) RNA was isolated from 40 μg of total RNA using oligo(dT)25 Dynabeads (Dynal,AS). The poly(A^+) RNA was eluted from the beads and first strand cDNA was synthesized using 200 units M-MLV reverse transcriptase and 0.5 μg oligo(dT) primer in 20 μl of the buffer recommended by the manufacturer. The reaction proceeded for one hour at 37°C and was stopped by heating to 95°C for five minutes. As a control for contamination with genomic DNA, 10 μg of RNA were digested with 1 U of RNase-free DNase for 15 minutes at 37°C prior to cDNA synthesis and PCR.

Six pairs of nested primers were selected according to the published COL4A5 sequence [7] (Table 1, Fig. 1). To allow for solid phase sequencing of the PCR products, one primer of each second set was biotinylated at its 5′ end. PCR was performed in 100 μl of 0.01% gelatine, 50 mM KCl, Tris-HCl 10 mM, 1.5 mM $MgCl_2$, 100 μM dNTP's, 2.5 units Taq DNA polymerase and 25 pM primer in the first PCR and 15 pM in the second.

One microliter of cDNA synthesis mix was used for the first PCR, which consisted of a five minute denaturation at 95°C and

five cycles of 45 seconds at 95°C, 10 minutes at 58°C, 1.5 minutes at 72°C, followed by 25 cycles of 45 seconds at 95°C, 45 seconds at 60°C and 1.5 minutes at 72°C. A total of 1.5 μl of the first PCR product was used in a second amplification for 30 cycles of 45 seconds at 94°C, 45 seconds at 60°C and 80 seconds at 72°C. Negative controls included PCR with water instead of the cDNA, with 2 μg of the original RNA sample and with 2 μg of the DNase-treated RNA sample, both without cDNA synthesis reaction. PCR products were first resolved on 1% agarose gels and visualized with ethidiumbromide. All samples were then sequenced [17] on an automated ALF (Pharmacia) sequencer using a solid phase approach and specific FITC-labeled primers.

Sequences were analyzed using the Intelligenetics Suite Ver 5.4 software.

DNA analysis

DNA was isolated from kidney samples using proteinase K followed by a phenol-chloroform extraction.

Two primers (F: 5'-TGTCGTCATTTGCTGTGGAT and R:5'-CATTTCCTACCTGGAGTCCT) flanking exon 10 were chosen according to the published sequence [18] generating an amplified fragment of 171 bp. The fragment was separated on a 0.7% agarose gel, purified using the Magic DNA Clean-up System (Promega), ligated into SmaI cut pGEM-3Z vectors and transformed into DH5α. Ten insert-containing clones were isolated and sequenced.

Results

Comparison of COL4A5 cDNA derived from lymphoblasts and kidney

The possibility to use mRNA isolated from lymphoblasts [19] to screen for COL4A5 mutations should allow a more comprehensive screening for mutations. Therefore, the amplification of 4664 bp of the COL4A5 cDNA starting from RNA isolated from EBV transformed white blood cells in six overlapping nested PCRs was optimized (Fig. 2). This covers part of the 7S domain and the complete triple-helical and carboxyterminal NC1 domains of the collagen α5(IV) molecule. The PCR products obtained from EBV transformed cells of a normal control were completely sequenced using a solid phase approach, and the sequence corresponded exactly to the one published by Zhou et al [7] proving amplification of the correct cDNA.

When the COL4A5 cDNA sequence was determined using the same strategy but starting with poly(A^+) RNA isolated from a kidney biopsy obtained from an Alport patient, two divergent regions were observed compared to the published consensus sequence.

First an additional 18 bp was observed between bp 3992 and 3993 of the published sequence [7]. A careful analysis of the fluorescent trace obtained by the automated sequencer also showed the additional presence of the consensus sequence (about 20 to 30% of the signal) (Fig. 3, bottom). The PCR and sequencing were repeated on a second cDNA sample obtained independently from the same RNA with identical results (not shown). The insertion of 18 bp occurred exactly at the boundary between two exons and conserved the reading frame of the COL4A5 mRNA. In addition the Gly-X-Y repeat of the collagenous triple helical domain was preserved by the insertion of two Gly-X-Y triplets. To exclude contamination with genomic

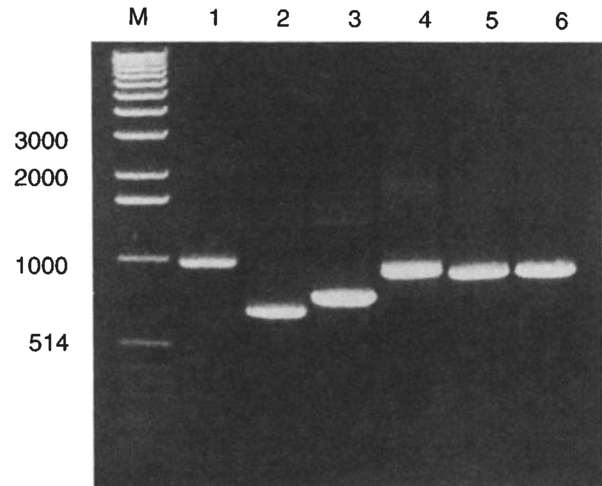


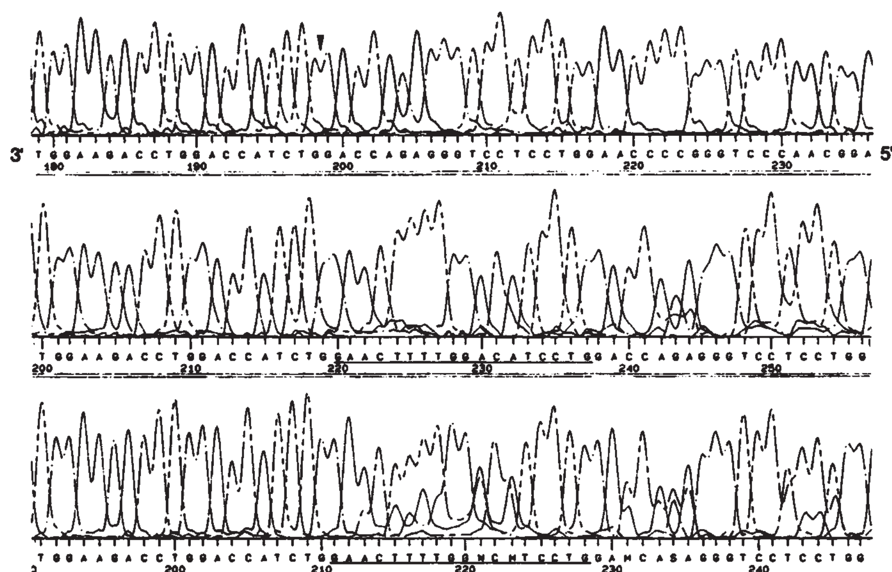
Fig. 2. *PCR amplification of the COL4A5 cDNA.* COL4A5 cDNA was synthesized from lymphoblasts mRNA and amplified in six overlapping fragments according the strategy outlined in Fig. 1. Abbreviation is M, size markers, with the size of the fragments indicated in bp at the left side of the picture. Lane 1 to 6: PCR products obtained by the amplification of the COL4A5 cDNA fragments 1 to 6, respectively.

DNA as a possible source of PCR artifacts, the experiment was repeated with DNase-treated RNA, with identical results. Furthermore, when the original RNA sample and the DNase-treated RNA sample were used as negative controls without prior reverse transcription, no PCR product was obtained (results not shown). Taken together, this strongly suggested that the insertion was a splice variant rather than a PCR artifact or a disease causing mutation. To analyze this further, RNA was isolated from an additional Alport kidney sample, three normal kidney samples and one cultured kidney cell sample, and the corresponding domain was amplified and sequenced. The COL4A5 sequence of these kidneys and the kidney cell culture showed an identical insertion of 18 bp (Fig. 3). This result was also confirmed by sequencing the PCR products generated by three other independent cDNA synthesis reactions and by sequencing the complementary strand (results not shown). Identical results were obtained when only one PCR was performed directly with the second set of primers on kidney cDNA, further excluding PCR artifacts. Sequencing of the same domain of the COL4A5 mRNA amplified from seven different EBV transformed cell lines and from four samples of white blood cells showed the consensus sequence (without the 18 bp insertion) in each of the cases.

Detection of a new complex AS mutation

In the second divergent region 7 bp (bp 4059 to 4065 of the sequence according to Zhou et al [7]) appeared to be deleted and three point mutations occurred (Fig. 4). The deletion of 7 bp changed the reading frame and introduced a premature stop codon 30 bp downstream of the mutation, resulting in a message for a truncated collagen α5(IV) molecule lacking the NC1 domain (Fig. 4). This sequence was also confirmed using a second independent cDNA sample. The remaining sequence corresponded exactly to the published COL4A5 sequence.

This complex mutation occurred in exon 10 (numbering



```
Deduced amino acid sequence        G P P G R P G                 L P G P E G
consensus nucleotide sequence   5' GGTCCTCCTGGGAGACCAG------------------GTCTACCAGGTCCAGAAGGT 3'
                                   |||||||||||||||||||                  ||||||||||||||||||||
Nucleotide sequence of             GGTCCTCCTGGGAGACCAGGTCCTACAGGTTTTCAAGGTCTACCAGGTCCAGAAGGT
kidney mRNA
deduced amino acid sequence        G P P G R P G P T G F Q G L P G P E G
```

Fig. 3. *Variant splicing of COL4A5 mRNA in kidney and white blood cells.* The COL4A5 cDNA sequence of EBV cells (upper), of a normal kidney tissue (middle) and of the kidney of an Alport patient (bottom) is shown. An 18 bp insertion between exon 10 and 11 codes for two Gly-X-Y triplets, conserves the reading frame of COL4A5 mRNA and is flanked by consensus splice signals. The boundary between exon 10 and 11 is indicated by a triangle; the 18 bp insertion is underlined. W = A + T; M = A + C. The sequence is read from 3′ to 5′.

```
            G  G  I  K  G  E  K  G  N  P  G  Q  P  G  L  P  G  L  P  G  L
A: 5'-(4035)GGAGGTATTAAAGGAGAGAAGGGAAATCCAGGCCAACCTGGGCTACCTGGCTTGCCTGGTTTGA
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B: 5'-(4035)GGAGGTATTAAAGGAGAGAAGGGAA--------CCTTCTTGGGCTACCTGGCTTGCCTGGTTTGA
            G  G  I  K  G  E  K  G  T          F  L  G  Y  L  A  C  L  V  *
```

Fig. 4. *A complex COL4A5 mutation detected in an Alport patient.* **A.** Wild type COL4A5 sequence with the amino acid sequence on top (numbering according to [7]). **B.** Sequence observed for the AS patient with the deduced amino acid sequence below. *The TGA stopcodon introduced by the frameshift.

according to Zhou et al [18]) of the COL4A5 gene. Since only a small needle biopsy (<10 mg) of the mother of the patient was available to investigate the origin of this complex mutation, a PCR was developed to amplify exon 10 of the COL4A5 gene. DNA was isolated from the kidney biopsy of the mother and exon 10 of the COL4A5 gene was amplified and subcloned. Ten clones were isolated containing a total of 12 copies of the PCR product. Sequencing detected six copies of wild type sequence and six copies of the mutant type sequence, indicating that the mother was heterozygous for a similar deletion in exon 10.

Discussion

Sequencing of the COL4A5 cDNA obtained by reverse transcription PCR of kidney poly(A^+) RNA revealed two divergent regions compared to the published sequence: an insertion of 18 bp and a complex deletion/missense mutation in exon 10.

The insertion shows all the properties of a variant exon: it is inserted at the boundary of exon 11 and 10 (numbering according to Zhou et al [18]), the reading frame is kept intact and the triple helical Gly-X-Y motif is conserved. The insertion is unlikely to be a PCR artifact: it was observed in five independent RT-PCR experiments with a normal kidney sample and with four additional kidney samples, and the inserted sequence is not derived from elsewhere in the COL4A5 cDNA. One round of PCR with second primers generated the same sequence further arguing against an artifact generated by the nested PCR. The 18 bp sequence is also not present in the published intron sequences 3′ of exon 11 and 5′ of exon 10 [18], suggesting that the observation is not the result of a partial splicing event. Identical results were obtained after DNase treatment of the RNA. PCR with the RNA or the DNase-treated RNA as a template, resulted in the absence of any PCR products showing the absence of any amplifiable template in the RNA samples. This, taken together with the fact that the remaining sequence corresponds exactly to the published

cDNA sequence also excludes amplification of genomic DNA as a source of this PCR product.

It should also be noted that within the mRNA sequence this 18 bp exon is flanked by consensus splice signals. This might explain the loss of this exon in the white blood cell mRNA. The published COL4A5 cDNA sequences were derived from placental and umbilical vein endothelial cDNA libraries [20] and a library derived from a fibrosarcoma cell line [7], respectively, and it is conceivable that in these sources a splicing pattern occurs comparable to the one observed in white blood cells.

An 18 bp exon would be unusually short in a collagen gene although non-fibrillar collagen genes do not follow the 54 bp rule for the exons coding for the Gly-X-Y repeats [21]. Thus far no reports were made of variant splicing of the COL4A5 gene and the significance of the insertion of two Gly-X-Y triplets on the association of α-chain monomers and on the cross linking of different triple helices is not known. It has been argued that for fibrillar collagens the triple helical domains have to be of the correct identical length whereas in the case of type IV collagens forming a flexible network this would be a less stringent requirement. However, in one Alport patient the skipping of exon 38 was reported leading to a deletion of nine Gly-X-Y triplets and this deletion was suggested to be directly related to the Alport pathogenesis in this patient [13]. Complex alternative splicing generates at least four different forms of α1(XIII) mRNA and interestingly this alternative splicing also involves exons coding for pure collagenous regions [22]. Differential splicing was also reported for the COL6A2 and the COL6A3 genes [23], but this affected only the globular aminoterminal and carboxyterminal domains of the type 6 collagen. Similarly splice variants were described modifying the aminoterminal domain of type II collagens [24].

Sequencing of this region of the COL4A5 cDNA of the kidney of the Alport patient reveals the presence of the consensus sequence in addition to the major signal of the insertion. This could be due to variant splicing within the same tissue, possibly influenced by the complex mutation observed in the exon downstream of the insertion (see further), or to the presence of a higher number of white blood cells in the Alport kidney. The observation of both sequences in two of four kidney samples could support the latter hypothesis.

Reverse transcription PCR based on the illegitimate transcription of genes in white blood cells is a powerful tool for the detection of mutations in a number of disease genes and it has been argued in a number of cases that the RNA processing was identical in the specific tissue and in the white blood cells [13, 19, 25]. The variant COL4A5 sequence, which is most probably the result of variant splicing, observed here in kidney compared to white blood cells demonstrates that the results obtained with white blood cell RNA should be interpreted with care, especially in the cases where no mutation is found.

The COL4A5 cDNA isolated from the kidney of the Alport patient shows a complex mutation formed by a 7 bp deletion and three nucleotide changes resulting in the generation of a premature stop and deleting part of the triple helical domain and the complete NC1 domain. This mutation was inherited from the mother. Deletions of the carboxyterminal part of the α5(IV) molecule were already reported in several Alport patients [26, 27], and it is suggested that these mutations result in more severe clinical manifestations compared to point mutations. This is in accordance with the finding of a rather early deafness and renal failure in the boy. The mother, however, is still having adequate renal function at the age of 42.

In conclusion, variant splicing of COL4A5 was found in the mRNA of kidney and white blood cells inserting a 18 bp exon between exon 10 and 11 in the kidney mRNA, and a complex mutation was detected in an Alport patient introducing a premature stop codon. The mutation was inherited from the mother.

Acknowledgments

This work was supported by a grant 'Geconcerteerde Acties' from the Belgium Government and by the Interuniversity Network for Fundamental Research sponsored by the Belgian Government (1991–1995). Peter Marynen is a "Bevoegdverklaard Navorser" of the "Nationaal Fonds voor Wetenschappelijk Onderzoek," Belgium.

Reprint requests to Prof. P. Marynen, Center for Human Genetics, Campus Gasthuisberg, O&N6, Herestraat 49, B-3000 Leuven, Belgium.

References

1. Atkin CL, Hasstedt SJ, Menlove L, Cannon L, Kirschner N, Schwartz C, Nguyen K, Skolnick M: Mapping of Alport syndrome to the long arm of the X chromosome. *Am J Hum Genet* 42:249–255, 1988
2. Flinter FA, Cameron JS, Chantler C, Houston I, Bobrow M: Genetics of classic Alport's syndrome. *Lancet* II:1005–1007, 1988
3. Brunner H, Schröder C, Van Bennekom C, Lambermon E, Tuerlings J, Mensel D, Olbing H, Monnens L, Wieringa B, Ropers H-H: Localization of the gene for X-linked Alport's syndrome. *Kidney Int* 34:506–510, 1988
4. Hostikka SL, Eddy RL, Byers MG, Höyhtyä M, Shows TB, Tryggvason K: Identification of a distinct type IV collagen a chain with restricted kidney distribution and assignment of the gene to the locus of X chromosome-linked Alport syndrome. *Proc Natl Acad Sci USA* 87:1606–1610, 1990
5. Zhou J, Barker DF, Hostikka SL, Gregory MC, Atkin CL, Tryggvason K: Single base mutation in α5(IV) collagen chain gene converting a conserved cysteine to serine in Alport syndrome. *Genomics* 9:10–18, 1991
6. Knebelmann B, Deschenes G, Gros F, Hors MC, Grünfeld JP, Tryggvason K, Gubler MC, Antignac C: 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, 1992
7. Zhou J, Hertz JM, Leinonen A, Tryggvason K: 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, 1992
8. Boye E, Vetrie D, Flinter F, Buckle B, Pihlajaniemi T, Hamalainen E-R, Myers JC, Bobrow M, Harris A: Major rearrangements in the α5(IV) collagen gene in three patients with Alport syndrome. *Genomics* 11:1125–1132, 1991
9. Antignac C, Zhou J, Sanak M, Cochat P, Roussel B, Deschenes G, Gros F, Knebelmann B, M.C. Hors-Cayla MC, Tryggvason K, Gubler MC: Alport syndrome and diffuse leiomyomatosis: Deletions in the 5′ end of the COL4A5 collagen gene. *Kidney Int* 42:1178–1183, 1992
10. Smeets JHM, Melenhorst JJ, Lemmink HH, Schröder CH, Nelen MR, Zhou J, Hostikka SL, Tryggvason K, Ropers HH, Jansweijer MCE, Monnens LAH, Brunner HG, Van Oost BA: Different mutations in the COL4A5 collagen gene in two patients with different features of Alport syndrome. *Kidney Int* 42:83–88, 1992

11. NETZER KO, RENDERS L, ZHOU J, PULLIG O, TRYGGVASON K, WEBER M: Deletion of the COL4A5 gene in patients with Alport syndrome. *Kidney Int* 42:1336–1344, 1992
12. M'RAD R, SANAK M, DESCHENES G, ZHOU J, BONAITI-PELLIE C, HOLVOET-VERMAUT L, HEUERTZ S, GUBLER M-C, BROYER M, GRUNFELD J-P, TRYGGVASON K, HORS-CAYLA M-C: Alport syndrome: A genetic study of 31 families. *Hum Genet* 90:420–426, 1992
13. NETZER K-O, PULLIG O, FREI U, ZHOU J, TRYGGVASON K, WEBER M: COL4A5 splice site mutation and α5(IV) collagen mRNA in Alport syndrome. *Kidney Int* 43:486–492, 1993
14. NOMURA S, OSAWA G, SAI T, HARANO T, HARANO K: A splicing mutation in the α5(IV) collagen gene of a family with Alport's syndrome. *Kidney Int* 43:1116–1124, 1993
15. HOSTIKKA SL, EDDY RL, BYERS MG, HÖYHTYÄ M, SHOWS TB, TRYGGVASON K: Identification of a distinct type IV collagen a chain with restricted kidney distribution and assignment of its gene to the locus of X chromosome-linked syndrome. *Proc Natl Acad Sci USA* 87:1606–1610, 1990
16. CHOMCZYNSKI P, SACCHI N: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
17. SANGER F, NICKLEN S, COULSON AR: DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA* 74:5463–5467, 1977
18. ZHOU J, HOSTIKKA SL, CHOW LT, TRYGGVASON K: Characterisation of the 3′ half of the human type IV collagen α5 gene that is affected in the Alport syndrome. *Genomics* 9:1–9, 1991
19. CHELLY J, HUGNOT JP, CONCORDET JP, KAPLAN JC, KAHN A: Illegitimate (or ectopic) transcription proceeds through the usual promoters. *Biochem Biophys Res Commun* 178:553–557, 1991
20. PIHLAJANIEMI T, POHJOLAINEN ER, MYERS JC: Complete primary structure of the triple-helical region and carboxyl-terminal domain of a new type IV collagen chain, α5(IV). *J Biol Chem* 265:13758–13766, 1990
21. SOININEN R, HUOTARI M, GANGULY A, PROCKOP DJ, TRYGGVASON K: Structural organization of the gene for the α1 chain of human type IV collagen. *J Biol Chem* 264:13565–13571, 1989
22. TIKKA L, ELOMAA O, PIHLAJANIEMI T, TRYGGVASON K: Human alpha 1(XIII) collagen gene. Multiple forms of the gene transcripts are generated through complex alternative splicing of several short exons. *J Biol Chem* 266:17713–17719, 1991
23. ZANUSSI S, DOLIANA R, SEGAT D, BONALDO P, COLOMBATTI A: The human type VI collagen gene, mRNA and protein variants of the α3 chain generated by alternative splicing of an additional 5-end exon. *J Biol Chem* 267:24082–24089, 1992
24. SANDELL LJ, MORRIS N, ROBBINS JR, GOLDRING MB: Alternatively spliced type II procollagen mRNAs define distinct populations of cells during vertebral development: Differential expression of the amino-propeptide. *J Cell Biol* 114:1307–1319, 1991
25. SARKAR G, SOMMER SS: Access to a messenger RNA sequence or its protein product is not limited by tissue or species specificity. *Science* 244:331–334, 1989
26. SMEETS HJM, MELENHORST JJ, LEMMINK HH, SCHRÖDER CH, NELEN MR, ZHOU J, HOSTIKKA SL, TRYGGVASON K, ROPERS HH, JANSWEIJER MCE, MONNENS LAH, BRUNNER HG, VAN OOST BA: Different mutations in the COL4A5 collagen gene in two patients with different features of Alport syndrome. *Kidney Int* 42:83–88, 1992
27. NETZER K-O, RENDERS L, ZHOU J, PULLIG O, TRYGGVASON K, WEBER M: Deletions of the COL4A5 gene in patients with Alport syndrome. *Kidney Int* 42:1336–1344, 1992