Alport syndrome associated with diffuse leiomyomatosis: COL4A5-COL4A6 deletion associated with a mild form of Alport nephropathy

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Abstract

Background. The X-linked Alport syndrome (AS) is an inherited nephropathy due to mutations in the COL4A5 gene, encoding the α5 chain of type IV collagen, a major component of the glomerular basement membrane (GBM). Here, we report a new kindred with the rare association of X-linked AS and diffuse leiomyomatosis (DL), which is a tumourous process involving smooth muscle cells of the oesophagus, the tracheobronchial tree and, in females, the genital tract. For this syndrome, an almost constant association of large COL4A5 rearrangements with a severe juvenile form of nephropathy has been described for male patients.

Methods. DNA rearrangement at the COL4A5-COL4A6 locus was studied in several members of this family using polymerase chain reaction and pulsed field gel electrophoresis. Furthermore, immunohistochemical staining of tumour and skin samples was performed.

Results. The affected patients in this family carry a 120 kb deletion by which the COL4A5 exon 1 and COL4A6 exons 1, 1’, and 2 are removed. Immunohistochemical investigation of a skin biopsy of an affected male patient confirmed the absence of both the α5 and the α6 chains of type IV collagen in the basement membrane of the skin. Surprisingly, both affected male patients had a rather mild renal phenotype.

Conclusions. This report shows that, contrary to what has been reported to date, patients suffering from AS associated with DL can be associated with a late onset renal failure (adult) form of nephropathy.

Keywords: adult type nephropathy; Alport syndrome; COL4A5-COL4A6 deletion; diffuse leiomyomatosis; DNA analysis

Introduction

Alport syndrome (AS) is an inherited nephropathy progressing to end-stage renal failure (ESRF), often associated with high-tone sensorineural deafness and specific eye signs (anterior lenticonus and macular flecks). It is characterized by abnormalities of the glomerular basement membrane (GBM) (thinning, thickening, and splitting) shown by electron microscopy. AS is due to mutations in one of three chains of type IV collagen that are highly expressed in the GBM: α3, α4, and α5(IV) [1]. These chains are encoded by three genes: COL4A3 and COL4A4 which are located head-to-head on chromosome 2 [2], and COL4A5 which is located on the long arm of the X chromosome, head-to-head with another type IV collagen gene, COL4A6. The latter encodes the α6(IV) chain that is not expressed in the GBM. Mutations of the COL4A5 gene, encoding the α5 chain of type IV collagen, are responsible for the most common (85%), X-linked form of the disease. In a few families, X-linked AS is associated with diffuse leiomyomatosis (DL), a tumourous process involving smooth muscle cells of the oesophageal wall, the tracheo-bronchial tree and, in females, the genital tract. A remarkable feature of AS associated with DL is a very severe nephropathy in males, rapidly progressing to ESRF early in life [3–8]. This is in agreement with the molecular defect underlying this association, resulting in null mutation of COL4A5 due to deletions removing the 5’-end of both the COL4A5 and COL4A6 genes.
In this paper we describe a new family affected by the DL-AS association. All affected individuals showed constitutional deletions removing the 5'-ends of COL4A5 and COL4A6. Surprisingly, both affected males in this family had a mild form of AS nephropathy.

Subjects and methods

Patients

We have assessed a Caucasian family with a history of nephropathy and oesophageal tumours, showing an X-chromosomal inheritance pattern (Figure 1). The clinical features could be traced back to the grandfather of the two index patients (patients 4 and 5 in Figure 1), who were operated at our hospital and provided the tissue samples for histological analysis in this study.

Their grandfather (patient 1) apparently suffered from dysphagia almost throughout his life. He died of renal failure aged 50. His daughter, the mother of the two index patients (patient 3), underwent surgery for an oesophageal tumour at the age of 30 after a 15 year history of dysphagia. No renal disease, visual, or hearing problems had been reported for her. Her son (patient 4) had an oesophageal resection of a histologically proven leiomyoma aged 13. Nine years later a tumour recurrence at the site of anastomosis was excised. He additionally suffered from hypoaacusis and was operated for bilateral cataracts. At the age of 40 he presented to our department with a second recurrence of his leiomyoma at the oesophagogastric anastomosis. Laboratory studies revealed a serum creatinine of 1.3 mg/dl and a creatinine clearance at 111 ml/min. A 8 x 5 cm sized solid tumour was detected with a contrast X-ray study and by CT-scan, and was then successfully enucleated in our hospital.

The 34-year-old sister (patient 5) reported a 20-year history of progressive dysphagia and heartburn on presentation. A CT-scan showed an 18 x 4 cm sized leiomyoma that caused a narrowing and shifting of the oesophagus as well as dystelectases of the right lung. Furthermore, an adenoma of the left adrenal gland, and hypertrophy of the clitoris were detected. She did not show any visual or hearing problems. Urinary and blood analysis revealed microhaematuria and proteinuria (350 mg/dl), a normal creatinine clearance (192 ml/min), and serum creatinine (0.57 mg/dl). The oesophageal tumour was excised by subtotal oesophagectomy followed by reconstructive colonic interposition. She was discharged without any symptoms.

Three of four daughters of patients 4 and 5 (patients 6–8) showed microhaematuria with normal renal function. Because of dysphagia all three of them had gastroscopy: patient 6 had already developed a 8 x 8 cm sized tumour of the lower oesophageal wall, patient 7 showed several small lesions, and patient 8 had a 3 x 2 cm sized solid tumour of the lower oesophagus. Patient 9 was not investigated because she was asymptomatic and still very young. After informed consent, blood samples were collected from six members of the family (patients 4, 5, 6, 7, 8, and 9).

Tissues and immunohistochemical analysis

Specimens of the oesophageal tumours were obtained from patients 4 and 5. Part of the tissue including a skin biopsy of patient 4, was snap-frozen in liquid nitrogen immediately after removal and stored at -70 °C. A representative section was cut from each tumour, fixed in formaldehyde, embedded in paraffin, and subjected to standard histopathological investigation. Further histochemical evaluation was performed to detect differentiated myogenic cells (alpha-smooth-muscle actin (ASMA); pan-muscle-specific actin (HHF 35)) and Schwann-cells (S-100 protein).

Immunohistochemical staining of the skin sample was done as previously described [9] using monoclonal antibodies recognizing the NC1 domain of the alpha 1 (MAB1, Wieslab AB, Lund, Sweden), the alpha 5 (MabA7, a gift from M. Kleppel [10], or of the alpha 6 chain (Mab H63, [11]).

DNA analysis

High-molecular-weight genomic DNA was prepared for pulsed field gel electrophoresis (PFGE) analysis from peripheral lymphocytes as described previously [5]. DNA was digested with NotI, NruI, SalI, and SfiI restriction enzymes, separated by PFGE, followed by hybridization with the appropriate DNA probes [5]. The probes used have been reported elsewhere: JZ4 and Pc4b are encoding the 5’- and the 3’-end of COL4A5 cDNA [12,13], respectively, and JZ3 is encoding the 5’-end of COL4A6 cDNA [14].

For polymerase chain reaction (PCR) analysis, DNA was extracted from peripheral blood leucocytes by a standard procedure. PCR amplification and agarose gel electrophoresis were carried out as previously described [5]. Primer sequences and annealing temperature are listed in Table 1. All PCR reactions were performed with 30 cycles of denaturation at 94 °C for 1 min, annealing at various temperatures (Table 1) for 1 min, and with extension at 72 °C for 1 min.

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**Fig. 1.** Pedigree analysis of the family affected with the DL-AS association: □ ○ = affected male/female (oesophageal leiomyoma, haematuria); □ ○ = non-affected male/female; † = patient died. No clinical information was available for the two sisters of patient 3.
Results

Histopathology

Histopathology of the resected oesophageal tumour of patient 4 showed spindle-shaped cells, formed in groups with nodular structures. Cells showed eosinophilic fibrillar cytoplasm and spindle-shaped nuclei. Neither mitosis nor higher-grade cell atypias were seen. Histological findings were consistent with the diagnosis of leiomyoma without malignant transformation. The resected oesophageal tumour of patient 5 showed similar findings, leading to identical diagnosis.

DNA analysis

In patient 4, we first studied DNA rearrangements using PCR amplification of genomic DNA. No amplification products were observed using primers flanking COL4A5 exon 1 (Figure 2), or a couple of primers located in COL4A6 exon 1 and COL4A6 exon 2, respectively. Conversely, COL4A6 exon 3 and the marker 909L (located in COL4A6 intron 3) were amplified. The same applies for primers amplifying the E7M13 marker located within COL4A6 intron 2 that was not deleted in this patient.

High-molecular-weight DNAs from six family members were analysed by PFGE. The Pc4b and JZ3 probes were hybridizing the same 830-kb NotI and 530-kb NruI junction fragments in male and female patients (Figure 3). Affected females also displayed normal sized restriction fragments (950 and 650 kb with NotI and NruI, respectively), whereas the control and the non-affected female (patient 9) only showed the normal fragment. All affected patients displayed a 60-kb SfiI junction fragment hybridizing the JZ4 probe (not shown). According to the map of the region, these results indicate a deletion of about 120 kb (Figure 4) removing COL4A5 exon 1, the intergenic region, and COL4A6 exons 1, 2, and 3.

Immunohistochemical analysis

The histochemical staining of the tumour samples showed a positive reaction for ASMA and HHF35, but...
no reactivity for S-100. The skin biopsy, taken from patient 4, showed normal \( \alpha_1(IV) \) chain immunoreactivity. There was, however, no immunoreactivity for \( \alpha_5 \) and \( \alpha_5(IV) \) chains in the basal membrane (not shown).

**Discussion**

In the present paper we studied DNA rearrangements in a family affected by the DL-AS association. Using PFGE and PCR analysis of genomic DNA, we showed that affected members of this family carry a 120 kb COL4A5-COL4A6 deletion removing COL4A5 exon 1 and COL4A6 exons 1, 1', and 2. This is in agreement with previous studies, which have shown that the extent of the deletions in COL4A6 in DL-AS patients is consistently confined to the first two exons of the gene [3,5,6], whereas patients with deletions extending further than COL4A6 exon 3 are not DL affected [5,7]. This reinforces the hypothesis that COL4A6 intron 2 contains unidentified crucial sequences involved in the regulation of smooth-muscle proliferation.

In X-linked AS, affected males develop ESRF at ages that vary widely from kindred to kindred, but run broadly through within a kindred. According to the severity of nephropathy, families can be classified in juvenile (age of ESRF in males <31 years) or adult (age >31) kindreds. Considering the many mutations identified in the COL4A5 gene, we [15], and others [16], have previously reported the almost constant association of large COL4A5 rearrangements with severe juvenile form of AS. Accordingly, in the DL-AS association, except of one case [17], all affected males reported to date [reviewed in 6,7] were suffering from a severe nephropathy. This is consistent with deletions observed in DL-AS patients, that are expected to lead to a complete absence of COL4A5 expression.

Surprisingly, in the DL-AS family we report here, both male patients did not present with renal failure at the ages of 40 and 50, respectively. DNA rearrangement analysis in this family showed COL4A5-COL4A6 deletions, which surely result into absence of the \( \alpha_5 \) chain of type IV collagen in GBM in affected male patients. Accordingly, immunohistochemical analysis of a skin biopsy of patient 4 showed no \( \alpha_5(IV) \) expression in the skin basement membrane. Both PFGE and PCR analysis strongly suggest that the deletions in this family were not associated with other types of complex rearrangements, such as duplication or inversion, which could have explained a rather mild kidney phenotype in males. Furthermore, the mutation was clearly inherited from patient 1, ruling out the possibility of a somatic mosaicism in patient 4, which has been previously reported in a DL-AS patient [8] and could have led to a less severe kidney disease.

We show that, similar to some COL4A5 point mutations, large deletions of the gene can result in the adult form of AS nephropathy. Whether other gene(s) may be implicated in determining the severity of the renal phenotype in COL4A5 null mutations remains to be examined. Our results indicate that the phenotypic expression of the renal disease in AS associated with DL is not only determined by the type of mutation, but also depends on other yet unknown mechanisms.

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