Alport syndrome and diffuse leiomyomatosis. Clinical aspects, pathology, molecular biology and extracellular matrix studies. A synthesis

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Résumé • Summary

L'association syndrome d'Alport-léiomyomatose diffuse peut être définie comme une maladie héréditaire du collagène de type IV associant les altérations d'un syndrome d'Alport (néphropathie hématurique, surdité et anomalies oculaires spécifiques : lenticone antérieur, maculopathie rétinienne) et une léiomyomatose œsophagienne, trachéobronchique et génitale (chez les femmes). Sa transmission héréditaire se fait selon le mode dominant lié au chromosome X. Les gènes responsables sont COL4A5 et COL4A6, (localisés, tête à tête, en Xq22), qui codent pour les chaînes α5 et α6 du collagène IV. Une délétion des parties 5' des deux gènes et de la région intergénique a été mise en évidence chez tous les malades. Le point de cassure dans COL4A6 est toujours situé dans l'intron 2. L'analyse immunohistochimique a montré d'importantes modifications de la composition des membranes basales des reins et des léiomyomes œsophagiens. Au niveau œsophagien, ces anomalies sont caractérisées par l'absence des chaînes α5 et α6 du collagène IV, de la fibronectine et de la chaîne β1 de laminine dans les membranes basales musculaires où elles sont normalement exprimées. Il s'y associe des anomalies des myocytes : expression irrégulière de la sous-unité α5 intégrine, et désorganisation des filaments d'actine et de desmine. Un troisième gène encore inconnu, localisé dans le large intron 2, dans une région critique de 90 kb délimitée par l'analyse des mutations, pourrait être responsable de la prolifération des cellules musculaires lisses. Les anomalies des membranes basales déstabilisant les interactions entre cellules et matrice extracellulaire pourraient également jouer un rôle pathogénique.

Mots clés : Syndrome d'Alport – Léiomyomatose – Léiomyomatose œsophagienne – Biologie moléculaire – Transmission liée au chromosome X – Expression des chaînes alpha IV.

The Alport syndrome-diffuse leiomyomatosis association can be defined as a hereditary disease of type IV collagen combining features of Alport syndrome (hematuric nephropathy, deafness and ocular abnormalities : anterior lenticonus, maculopathy) and leiomyomatosis involving oesophagus (diffuse type), tracheobronchial tree, and genitals (only in women). This entity is transmitted as an X-linked dominant trait. Mutations of both the COL4A5 and COL4A6 genes, located head to head in Xq22 encoding the α5 and α6(IV) chains are responsible for the abnormalities. Molecular studies have shown deletions of the 5' end of both COL4A5 and COL4A6 including the intergenic region. The breakpoint in COL4A6 is always located within intron 2. Immunohistochemistry has shown significant alterations of basement membranes in the kidney and esophageal leiomyomas. Leiomyomas lack α5 and α6(IV) chains, fibronectin and laminin β1 chains in the muscle basement membranes where they are normally expressed. The tumors also show myocyte anomalies : irregular expression of the α5 integrin subunits, and disorganization of actin and desmin filaments. It is hypothesized that a third as yet unknown gene, situated within the large intron 2 in a critical 90 kb region, is responsible for the smooth muscle proliferation. Abnormalities of the basement membranes could destabilize interactions between muscular cells and the extracellular matrix.

Key words : Alport syndrome – Leiomyomatosis – Oesophageal leiomyoma – Molecular Biology – X-linked transmission – Expression of alpha IV chains.

Alport syndrome and diffuse leiomyomatosis (AS-DL) is a rare association, first described as a new syndrome in 1983[1]. Since then, more than 40 cases have been reported in the medical literature. In affected families, cosegregation of the two pathologies has been observed in all male and most female patients, making a fortuitous association very unlikely[2].

This association is defined as the combination of Alport syndrome (AS) features (hematuric nephropathy, deafness and

ocular abnormalities : anterior lenticonus, maculopathy) and leiomyomatosis involving oesophagus (diffuse type), tracheobronchial tree, and genitals (only in women).

The first 35 cases (21 women, 14 men) were reviewed by Antignac[3]. Fifteen were adults and 12 children ; the age was not reported in 8. The disease is generally expressed during childhood, although it can be manifested only in adulthood. Any patient with AS should be examined for signs of diffuse leiomyomatosis (DL) and vice versa[4].

Clinical manifestations

Hematuric nephropathy which is progressive in men and non-progressive in women was a common finding. All male patients presented the severe, juvenile type disease. Nine reached end-stage renal disease (ESRD) at around 20 years of age. The 4 remaining patients (age 4 to 9 years) with available clinical data had microscopic hematuria (detected at birth in two and at three years of age in the two others). Out of the 19 women with available data, 16 had isolated microscopic hematuria. Interestingly, a 39-year-old female patient with severe DL had no renal symptoms and normal renal biopsy, whereas her daughter had hematuria[2, 3].

Progressive high-tone deafness in men is also a common symptom. In Antignac's review[3], eight out of nine male patients with ESRD were deaf and two out of four children under nine years of age with only microscopic hematuria had high tone hypoacousia. None of the affected women had hypoacousia.

Regarding ocular abnormalities, it is worth noting that more than one third of the reported patients (7 men, 6 women) had congenital bilateral cataracts[3]. This conspicuous feature of the syndrome is not commonly found in AS without DL. Anterior lenticonus was present in three male children, and macular flecks in one of them, both of which are typical of AS without DL. Eye abnormalities accounted for blurry vision, diminished visual acuity and myopia.

Leiomyomatosis was of course present in all cases. All had diffuse oesophageal involvement leading to dysphagia, odynophagia, retrosternal or epigastric pain, regurgitation and post-prandial vomiting. Bleeding has occasionally been reported[1-3].

Tracheobronchial leiomyomatosis was reported in 4 cases after autopsy, one of them with a pediculated leiomyoma of the carina. Patients with these abnormalities may have recurrent bronchitis, bronchiospasms simulating bronchial asthma, dyspnea, cough, stridor and apnea[1-3].

Genital leiomyomata were observed in 9 out of 14 adult women. The location was variable : uterine, vulvar, perivaginal, periurethral and perirectal, as well as clitoral hypertrophy. Signs and symptoms were also variable, depending on the location and size of the tumors[2, 3].

Pathology

On light microscopy, renal biopsies and autopsies of patients with the AS-DL association displayed irregular and non-specific glomerular changes with increased extracellular matrix, especially in cases with long duration of the disease. Irregular and non-specific lesions were also observed in the other histological structures of the kidney[1-3].

Electron microscopy (EM) studies repeatedly showed the typical ultrastructural abnormalities of glomerular basement membranes (GBM) seen in AS alone, consisting of alternate segments of thickening and thinning of the GBM with splitting and fragmenting of the lamina densa into several strands forming a « basket-weave » pattern ; silver stains demonstrated duplications and irregularities along the GBM[1-3].

The abnormal proliferation of smooth muscle was constituted by diffuse hyperplasia and/or multinodular leiomyomata. It was mainly represented by a diffuse leiomyoma of the oesophagus whose typical presentation was a tumor process involving all muscular layers. It was typically located in the lower part of the oesophagus, in some cases spreading to the upper part of the stomach ; occasionally, it involved the entire oesophageal muscular wall extending from the pharyngo-oesophageal to the gastro-oesophageal junctions. Diffuse hyperplasia of smooth muscle fibers were also found in the bronchiolar tree, the membranous portion of the trachea, and the female genitals (clitoris, vulva, vagina, uterus). The histological characteristics of all these lesions were those of benign proliferation of smooth muscle cells, without atypical nuclei or abnormal mitosis[1-3]. Myocyte cytoplasm showed central areas of rarefaction which were actin-positive and desmin-poor, with the reverse pattern of staining at the cell periphery[5]. In one case of diffuse oesophageal leiomyoma examined by EM, thickening and duplication of basement membranes (BM), alternating with focal thinning or total absence of this structure in some segments were observed[5].

Hereditary transmission

Examination of family pedigrees confirmed X-linked dominant transmission with variable expressivity. Penetrance for nephropathy was 100% in men and 80% in women, and that for leiomyomatosis was 100% in both sexes. Penetrance for cataracts was incomplete in both sexes. Reported sporadic cases probably represent de novo mutations[2, 3, 6].

Molecular biology

Type IV collagen is the major component of BM. Collagen IV, like other collagens, is constituted by monomers. A monomer is a triple helical molecule composed of three α chains. Six distinct α chains, 1 to 6(IV), have been identified and each one is encoded by a different gene. These genes are located in pairs on three different chromosomes. Each pair is situated in a head to head arrangement, with an intergenic region containing a common promoter which acts bidirectionally[7].

The α1(IV) and α2(IV) chains are encoded by the COL4A1 and COL4A2 genes respectively, located on chromosome 13 region q34. They have a ubiquitous distribution and are found in all BM ; thus their absence is considered lethal in utero. Interestingly, in the glomerular tuft these chains are expressed strongly in the mesangial region but faintly in the peripheral capillary wall, where they are located within the subendothelial aspect of the BM[8-10]. Alpha 3 to 6(IV) chains have a restricted distribution. The α3 and α4(IV) chains (encoded by COL4A3 and COL4A4 respectively, located on chromosome 2q35) are codistributed in the glomerular and distal tubule BM, as well as in lung alveolar BM and specialized BM of eye and internal ear[8-10]. Alpha 5(IV) and α6(IV) chains are encoded by COL4A5 and COL4A6 respectively, located on chromosome Xq22. The α5(IV) chain has the

same distribution as α3 and α4(IV) but is also present in collecting duct, epidermal and oesophageal smooth muscle cell BM[9-11]. The α6(IV) chain is codistributed with the α5(IV) in most BM, except in the GBM where it is absent[11].

Mutations of the COL4A5 gene cause X-linked Alport syndrome[7, 12, 13], while deletions of both COL4A5 and COL4A6 are responsible for the Alport syndrome-diffuse leiomyomatosis association[7, 14-17]. To date, molecular analyses performed on more than 15 patients with AS-DL have shown deletions of the 5' end of both COL4A5 and COL4A6 including the intergenic region. The extension of the deletion in COL4A5 is variable and may be short (involving only the first exon) or very long (including the whole gene). In contrast, deletions in the COL4A6 gene are confined to the first two exons, the breakpoint being always located within the large intron 2, in a 90 kb critical region. Recently, it has been shown that the occurrence of the large deletions giving rise to AS-DL was favored by the presence of LINE-1 repetitive elements[18].

Interestingly, four patients with AS and no evidence of DL had deletions affecting the 5' end of COL4A5, extending beyond exon 3 in COL4A6[16]. The deletion removed the entire COL4A6 gene in one patient. Thus, the complete deletion of this gene does not cause smooth muscle proliferation. It has therefore been hypothesized that the tumor process is induced by the synthesis of a truncated α6(IV) chain or alternatively by the disruption of another as yet unidentified gene, lying in that critical region within intron 2. A deletion of part of this hypothetical gene would cause enhancement or lack of inhibition of smooth muscle proliferation.

In situ hybridization

In 1995, a COL4A6 transcript which included exon 4 but not exon 3 was detected by reverse transcriptase-PCR in the tumor sample of a male patient with AS-DL, using two different RNA probes corresponding to the collagenous and non-collagenous domains of COL4A6[16]. The expression of this transcript was confirmed by in situ hybridization revealing strong RNA expression in four oesophageal tumors from patients with the association AS-DL[19]. Thus, this COL4A6 transcript was expressed by myocytes despite the deletion mentioned above. Such a transcript could have a still unknown role in the proliferation of muscular cells.

Immunohistochemistry

Significant advances have been recently made in the understanding of the expression of the mutated genes in the extracellular matrix of the oesophagus and the kidney. Heidet et al.[19] used antibodies directed against protein components of the BM and integrin subunits in the normal fetal and mature oesophagus, as well as in hereditary DL. Results demonstrated that α5(IV) and α6(IV) chains were absent from smooth muscle cell BM of leiomyomas despite the expression of the α6(IV) transcript previously found by in situ hybridization. Such results rule out the hypothesis of the truncated α6(IV) chain as a cause of DL. In addition, fibronectin and β1 laminin chains were also missing and the α5 integrin subunit was unevenly expressed. Thus, in hereditary DL, significant changes in the composition of the extracellular matrix alter the expression of integrin receptors[19].

Similar abnormalities have been recently documented by immunohistochemistry in nine sporadic oesophageal leiomyomas from five males and four females. Deletion of COL4A5 and COL4A6, similar to that observed in the AS-DL association, has been found in the frozen leiomyoma sample from one of these patients who did not have constitutional mutation, demonstrating that this deletion resulted from somatic mutation[20].

A pathogenic hypothesis for DL was recently proposed by Thorner et al.[5] on the basis of ultrastructural and immunohistochemical studies of one AS-DL patient. According to the authors, the mutation in COL4A5 and COL4A6 induces successive events starting with specific BM abnormalities, leading to disruption of the normal cytoarchitecture through the loss of integrin α5β1 or proteins interacting with this molecule. This would result in smooth muscle proliferation due to missignaling between extracellular matrix and cytoskeleton. However, this hypothesis does not account for the absence of smooth muscle cell proliferation in patients with large COL4A6 deletion.

In kidney and epidermal BM from most male patients with X-linked AS, the α3, α4 and α5 chains of type IV collagen are absent whereas discontinous (mosaic) distribution is observed in females[9-10]. The α1(IV) and α2(IV) chains are overexpressed especially in the glomerular BM in the early stages of the disease[9-10, 21]. In advanced stages, collagen type I, V and VI are overexpressed in mesangiocapillary localizations, whereas laminin, α1(IV) and α2(IV) are diminished or absent[10, 21, 22].

Although the rearrangements of extracellular matrix in X-linked AS described above are the most common features reported to date, other immunohistochemical patterns have been described and a correlation with the type of mutation has been made in some patients. Complete absence of α3(IV) to α6(IV) within skin and renal BM was observed in all male patients with large deletion, nonsense or frameshift mutation[13, 23-25]. Conversely different patterns including normal, weak antigenicity, or absence of the α3-6(IV) chains in the GBM have been seen in patients with missense mutations[23-25]. Patients with splice site mutation showed complete absence or normal expression of these chains[13, 24].

In conclusion, AS-DL is a contiguous gene syndrome involving COL4A5 and COL4A6 genes, and transmitted as an X-linked dominant trait. Large deletions identified in all patients affected with the syndrome allows genetic counselling and prenatal diagnosis. However the basis for smooth muscle cell proliferation in the syndrome is still unknown. A current hypothesis is that DL could result from disruption of the COL4A6 gene or of an as yet unidentified gene lying in the second intron of COL4A6 and behaving as a tumor or proliferation suppressor gene. The same genetic mechanism seems to be operative in isolated oesophageal leiomyoma. Several laboratories are actively pursuing further investigation to characterize the DL causing gene. Its identification is expected to lead to significant progress in the understanding of the pathophysiology of smooth muscle cell proliferation.

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