Letters

A family with X-linked Alport syndrome confirmed by skin biopsy

Sir,

Type-IV collagen is a major structural component of basement membranes. Six type-IV collagen chains, \(a_1(IV)\) to \(a_6(IV)\), have been identified [1]. The \(a_1(IV)\) and \(a_2(IV)\) chains are present in all basement membranes. The \(a_3(IV), a_4(IV), a_5(IV),\) and \(a_6(IV)\) chains are expressed selectively in the basement membranes of some tissues, such as kidney, cochlea, and eye.

Alport syndrome (AS) is a hereditary nephritis caused by defects in type-IV collagen in glomerular basement membranes (GBM), and it is characterized by a progressive glomerulonephritis associated with extrarenal features including auditory and ocular abnormalities [1,2]. AS is classified as X-linked (XL) form, autosomal recessive (AR) form, and autosomal dominant (AD) form. The XL form is associated with mutations in the COL4A5 gene [2]. The AR form is caused by COL4A3 or COL4A4 gene mutations [2]. Genetic mutation detection has become the ultimate and sufficient diagnostic criterion of AS. However, gene analysis in patients with AS remains a tedious and sometimes unsuccessful task because the incriminated genes contain more than 50 exons and there are no hot spots. In the meantime, the study of skin biopsy provides valuable diagnostic information in some patients with XL-AS, because the epidermal basement membrane (EBM) normally contains \(a_5(IV)\) [1–3]. This method is easier than gene analysis.

Here we report a family with XL-AS, which was confirmed by skin biopsy. We also discuss about extrarenal abnormalities and histological differential diagnosis in our patients.

Cases. Case 1, a 19-year-old Japanese man, was admitted to our hospital for initiation of the administration of continuous ambulatory peritoneal dialysis (CAPD). He was the only child of case 2. His maternal grandmother was diagnosed as having chronic glomerulonephritis, but a renal biopsy was not performed. At the age of 2, he underwent a renal biopsy because of persistent haematuria and proteinuria. Light microscopy showed diffuse mild mesangial proliferative glomerulonephritis. Immunofluorescent studies demonstrated no staining for immunoglobulins or complement. Electron microscopy disclosed diffuse thin GBM. At this time, he was diagnosed as having non-IgA glomerulonephritis with thin GBM. His renal function gradually decreased, and reached end-stage renal disease (ESRD) 14 years later. Keratoconus was observed, but a hearing defect was not detected by an audiogram.

Case 2 was a 43-year-old Japanese woman. At the age of 29, she received a renal biopsy because of persistent haematuria and proteinuria with mild renal impairment. Light microscopy showed diffuse mild to moderate mesangial proliferation and segmental sclerosis. Immunofluorescent studies demonstrated \(2+\) fine granular staining for IgA and \(1+\) granular staining for C3 over the mesangial area and along the GBM. Electron microscopy revealed diffuse thickening and irregularities of GBM with electron dense deposits along GBM and over the mesangial area. At that time she was diagnosed as having non-active IgA glomerulonephritis. However, her renal function gradually decreased, reaching ESRD 12 years later. She had neither eye abnormalities nor hearing loss. From their family history, clinical course, and histological findings, we suspected that the family had XL-AS. The frozen renal tissues were not available for staining \(a_5(IV)\) chain because of their poorly
preserved condition. We therefore performed skin biopsies to detect the defect of α5(IV) chain.

Skin tissues obtained from one healthy subject and the two patients were placed in OCT compound (Sakura Finetechnical Co., Ltd, Tokyo, Japan) and snap-frozen in −80°C. They were cut into 4-μm sections in a cryostat and air dried at room temperature. The sections were washed with phosphate-buffered saline (PBS, pH 7.4), and they were incubated with a mixture of Texas-red-conjugated monoclonal antibody against α2(IV) and fluorescein isothiocyanate-conjugated monoclonal antibody against α5(IV) (Shigei Medical Research Institute, Okayama, Japan) for 1 h at room temperature. After washing with PBS, they were mounted and examined using a Zeiss microscope. The antibody against α2(IV) stained EBM in a linear pattern in both a healthy subject and the patients (Figure 1A, C, and E). Although the antibody to α5(IV) showed staining similar to α2(IV) in the normal skin (Figure 1B), the son was completely negative for α5(IV) (Figure 1D) and the mother showed a mosaic staining pattern (Figure 1F). From these findings, we finally diagnosed the patients as having XL-AS.

Comments. The XL-AS usually affects males more severely than females [1–3]. Affected males generally develop ESRD before 31 years of age. In affected females, the wide range of severity which is observed is reported to be due to the well-known random inactivation of one of the X chromosomes [2]. The probability of developing ESRD in female patients is 12% by age 40 [2]. Reported risk factors for ESRD include a history of gross haematuria in childhood, nephrotic syndrome, and diffuse GBM thickening [2]. Since case 2, the mother, had diffuse GBM thickening, she developed ESRD by the age 40.

It is known that patients with AS often have extrarenal abnormalities such as sensorineural hearing loss and eye abnormalities [1,2]. Sensorineural hearing loss is observed in 30–40% of the patients, and deafness usually becomes symptomatic before the onset of ESRD. However, some families do not suffer from hearing loss. Our patients also have no hearing defect. It is therefore important for physicians to recognize these facts, because we cannot exclude the possibility of AS even if patients with unexplained CRF or persistent haematuria and proteinuria have normal hearing.

Renal morphological features in patients with AS by light microscopy are varied and non-specific [2,4]. In the early stages of the disease, some patients have no abnormalities. In the later course of this disease, mesangial proliferation, hypertrophy of podocytes, and segmental irregularities of GBM, segmental sclerotic lesions, and glomerular obsolescence are observed. Immunofluorescent studies have usually been non-specific, or negative in most patients. In contrast, electron microscopy shows characteristic changes including

Fig. 1. Immunohistochemistry of skin biopsies. Sections were stained with monoclonal antibodies against α2(IV) (A, C, E) and α5(IV) (B, D, F). (A and B) Skin from a normal subject; (C and D) skin from a male (son) with X-linked Alport syndrome; (E and F) skin from a female (mother) X-linked Alport syndrome. No staining of α5(IV) chain was observed in the epidermal BM of the male patient. A mosaic pattern of α5(IV) chain was noted in the epidermal BM of the female patient.
thickening and splitting of GBM. However, some patients have GBM of normal structure or thin GBM in the early stages of this disease [5]. In our case 1, electron microscopic findings also demonstrated diffuse thin GBM. This finding may reflect the early course of AS in this patient. On the other hand, we had initially considered the mother as having IgA nephropathy based on predominant IgA deposits in the glomeruli, especially along the GBM, although diffuse thickening and irregularities of GBM suggested the diagnosis of AS.

In normal skin, EBM contains $\alpha_1$(IV), $\alpha_2$(IV), $\alpha_5$(IV) and $\alpha_6$(IV), and normal GBM consists of $\alpha_1$(IV)–$\alpha_5$(IV), but not $\alpha_6$(IV) [1–3]. The expression pattern of $\alpha_5$(IV) is the same in EBM and GBM. Therefore, the defect of $\alpha_5$(IV) in patients with XL-AS, which is caused by mutations on COL4A5 gene, can be detected by skin biopsy. The skin biopsy is less invasive than renal biopsy. The sensitivity of the test is about 75% among patients with XL-AS [2]. The defect of $\alpha_5$(IV) is specific for the diagnosis of XL-AS, although the normal presence of $\alpha_5$(IV) in EBM is occasionally observed in XL-AS patients with point mutation. In latter cases, genetic analysis provides the conclusive diagnosis of AS.

In conclusion, we must consider the possibility of AS in patients with unspecified chronic glomerulonephritis but with suspicious clinical and pathologic features. Skin biopsy should be performed in such patients to detect the absence of $\alpha_5$(IV), since skin biopsy is less invasive than renal biopsy, and it can be performed in patients with CRF when renal tissue cannot be obtained because of renal atrophy.

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