Immunohistochemical Study for the Diagnosis of Alport's Syndrome

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Background: *Alport's syndrome (AS) is the most common cause of inherited glomerular disease in Thailand. The majority of cases show X-linked inheritance, which is caused by mutations in the gene coding for the α5 chain of type IV collagen in the glomerular basement membrane (GBM) and epidermal basement membrane (EBM). Such mutation usually leads to a reduction in protein amount, thus, immunohistochemical studies have been considered in diagnostic evaluation.*
Objective: *To study the expression of α[IV] collagen chains in the skin as an alternative approach to diagnose AS.*
Material and Method: *Eleven unrelated probands with proven AS, 7 relatives with abnormal urinalysis, 4 suspected individuals, and 8 normal controls were enrolled. A punch skin biopsy and immunofluorescence staining of the tissue specimens for α1, α3 and α5[IV] collagen chains was performed.*
Results: *The α5[IV] chain was absent in the EBM in all male AS patients while a discontinuing pattern was observed in all females except one. The findings are specific for AS with a sensitivity of 91%. Studies in relatives and suspected individuals also confirmed the advantage of this approach as demonstrated by the absence and discontinuation of α5[IV] staining in all males and females, respectively. We also analyzed their expressions in the kidney tissue and demonstrated abnormal α3 and α5[IV] staining in five of six samples.*
Conclusion: *Immunohistochemical study of the skin should be used as a screening method in patients suspected of AS, as it is much less invasive. Moreover, it is a useful adjunct to conventional examination of biopsied renal tissue.*

Keywords: *Alport's syndrome, Hereditary nephritis, Collagen type IV, Immunohistochemistry*

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Alport's syndrome (AS) is an inherited disorder of the glomerular basement membrane (GBM) due to abnormalities in type IV collagen, the main collagenous constituent of all basement membranes. It is the most common form of hereditary nephritis with progressive renal failure encountered worldwide and in our country[1]. AS occurs predominantly in men, and affected patients classically have persistent glomerular hematuria, proteinuria (usually in the subnephrotic range), progressive renal insufficiency, and high tone sensorineural hearing loss. Lenticonus of the anterior lens capsule, retinal anomalies, and leiomyomatosis are sometimes associated with the disease[2].

Type IV collagen in the GBM is composed of three · chains that form a triple helical molecule. Six genetically distinct type IV collagen chains, α1[IV]

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through α6[IV], have been identified. They are encoded by distinct genes (designated *COL4A1* to *COL4A6*) arranged pair-wise on three different chromosomes: *COL4A1* and *COL4A2* on chromosome 13, *COL4A3* and *COL4A4* on chromosome 2, and *COL4A5* and *COL4A6* on the X chromosome. In approximately 80% of AS pedigrees, the disease is X- linked and mutations identified so far are in the *COL4A5* gene (OMIM #305010). Affected males with X-linked AS have more severe phenotypes and usually progress to end stage renal disease, whereas heterozygous females have more variable phenotypes ranging from asymptomatic hematuria to renal insufficiency, and 10 percent of patients have no hematuria. About 5 percent of AS pedigrees showed an autosomal recessive pattern of inheritance (AR-AS) of which mutations are identified in the *COL4A3* and *COL4A4* genes (OMIM #203780), and the rarely found autosomal dominant form has been reported in a few kindreds (OMIM #104200). Up to 15 percent of new AS patients in the western countries have no family history suggesting that novel mutation might be common[3].

Conventionally, definite diagnosis of AS has been made on the basis of histological and ultrastructural examinations of the biopsied renal tissue from patients with glomerular hematuria and positive family history. Nonetheless, the diagnosis in some patients was made solely from renal tissue examination without extrarenal features and family history, Diffuse thinning of GBM may be the only finding in some cases, particularly early in the course, whereas characteristic changes of multi-lamellation or diffuse thickening and splitting GBM are found in adult patients. The latter findings, though, are only highly suggestive but not proven to be pathognomonic for AS since analogous changes may be found in other immune-complex glomerulonephritides[4]. Given the currently available information of the genes encoding all type IV collagen α chains (*COL4A1-COL4A6*), it is possible to use mole-cular genetic analysis to diagnose patients with AS. This study remains a challenge because of the hetero-geneity of locus and allele as well as the huge size of coding gene and exons to be screened. Moreover, it is still costly and not readily available in most places in our country.

In view of the fact that mutations in AS typically lead to a reduction in the protein amount, accessibility of specific monoclonal antibodies against all type IV collagen α chains yielded a chance to study the expression in the basement membrane of AS patients. Normal expression of each type IV collagen α chains has been thoroughly studied by indirect immunohistochemical techniques in several human tissues including the kidney, eye, cochlea, lung, brain, and skin[2,5,6]. In the kidney, the α1[IV] and α2[IV] chains are present in all basement membranes and, within the glomerulus, are restricted to the mesangial matrix and the subendothelial aspect of the GBM. The α3[IV] and α4[IV] chains are present predominantly in the GBM, occasionally in the distal tubular basement membrane (TBM) and in segments of the Bowman's capsule. The α5[IV] chain is present in a linear fashion in the GBM, Bowman's capsule, distal tubules and collecting ducts while the α6[IV] chain is located in the basement membrane of Bowman's capsule, distal tubules and collecting ducts. In the skin tissue, the expression of α1, α2, α5 and α6 chains, but not α3 and α4, of type IV collagen is observed in the epidermal basement membrane (EBM).

In patients with X-linked AS, the immunohistochemical studies generally revealed absent or discontinuous staining in particular basement membrane for α5[IV] as a result of mutations of the *COL4A5* gene[7-11]. As for AR-AS, mutations of the *COL4A3* or *COL4A4* gene also result in the loss of the α3-α4[IV] chain in the GBM[12]. Based on an extensive critical review of the literature, skin biopsy is the most useful and sensible approach for diagnostic screening since skin tissue is apparently easy to access and it is less invasive than renal biopsy[7,8,10]. This method is of value in identifying patients with AS or even for detecting asymptomatic female carriers who have isolated hematuria and hesitate to have a renal biopsy done. To our knowledge, skin immunohistochemistry for diagnosis of AS has never been performed in our country.

In this study, we developed an immunohistochemical technique to analyze the expression of type IV collagen α chains in the skin and renal tissue in previously diagnosed AS patient and relatives. The primary objective was to examine the sensitivity and specificity of using skin biopsy for the diagnosis of AS in Thai patients, and its correlation with renal abnormalities and other clinical parameters. We also studied the pattern of type IV collagen α chain expression in the skin tissue of suspected individuals, and in the renal tissue of patients previously diagnosed as AS by conventional examination using electron microscopy.

Material and Method

Subjects

We reviewed the medical and histopathological reports in those patients whose prior diagnosis of

AS was made at Siriraj Hospital from 1985 to 2005. The patients were divided into two groups: (1) Definite AS with a characteristic pattern of the GBM changes, i.e. diffuse irregular thinning/thickening and multi-lamellation, and positive family history of nephritis, hearing loss or ocular abnormalities in the first-degree relatives, and (2) Highly suggestive AS with characteristic pattern of the GBM changes without family history.

The tissue specimens used in this study included:

1. Non-fixed renal biopsied tissue from the patients (N = 6) and various types of acquired minor glomerulopathies (as positive control for GBM staining, N = 8).

2. Punched skin biopsied specimens from the patients (N = 11), their first-degree relatives (N = 7), individuals suspected of having AS (N = 4), and those obtained from normal tissue surrounding nevus or warts (as a positive control for EBM staining, N = 8).

Immunohistochemical procedures

The monoclonal antibodies against type IV collagen α chains were made available from Wieslab (Lund, Sweden). The antibody panel including monoclonal antibodies of the α1 chain (MAB1), the α3 chain (MAB3), and the α5 chain (MAB5) was used for indirect immunofluorescent studies of both skin and renal tissue.

Skin specimens (~ 4 mm in diameter) were obtained from the volar aspect of the forearm by punch biopsy, snapped frozen in liquid nitrogen, and stored at -70°C until used. Non-fixed renal tissue previously obtained by percutaneous needle biopsy and stored at -70°C was used. The tissue was cut into 3 μm-section in a cryostat, air-dried and fixed in acetone for 10 minutes. After hydration in phosphate buffer saline (PBS) buffer pH 7.4, the tissue was pre-treated with glycine/urea solution (0.1 M glycine, 6 M urea pH 3.5) for 10 min, rinsed with distilled water, blocked with mouse sera, and incubated in a moist chamber with the appropriate dilution of primary antibodies[13]. After 1 hour of incubation, the sections were washed three times with PBS (5 min each), and an appropriately diluted FITC- (for skin tissue) or Cy3- (for renal tissue) tagged secondary anti-mouse antibodies were applied. The sections were incubated for an additional 1 hour at room temperature, washed three times with PBS, and mounted in Vectashield (Vector Laboratories, CA). The sections were examined under fluorescence microscopy. For proper interpretation, a negative control (without primary antibody) and a positive control (α1[IV] staining) were run alongside. Three independent investigators judged all tissue sections and photographed them in a similar condition.

All procedures were performed under permission of the patients or close relatives after the risks and benefits of the studies had been explained. This study was performed with the prior approval of the Siriraj Hospital Committee on the Use of Human Subjects in Research.

Statistical analysis

Data was analyzed using the Software Package for Social Sciences (SPSS) 13.0 for Windows (Chicago, IL). Means, standard deviations, and percentages were used as descriptive statistics where appropriate.

Results

Eleven patients with renal biopsy indicative of AS belonging to 10 unrelated families from 1985 to 2005 were included in the study. Ultrastructural changes of the GBM as observed by electron microscopy consisted of irregularity in thickness, splitting, and generalized thinning with focal splitting. Family history of renal disease was documented in eight of ten probands. Thus, the diagnosis of AS is considered as definite in nine and highly suggestive in two patients. Sensorineural deafness, particularly high frequency loss, was observed in five patients, while anterior lenticonus was only presented in one patient (PY-II:7). The mean age at diagnosis was 11.3 $\pm$ 7.6, range 3-27 years. The ratio of male to female was 9:2. Recurrent gross hematuria was observed in eight patients while microscopic hematuria was found in three. None of the patients presented with nephrotic syndrome. Initial azotemia as defined by serum creatinine greater than 1.5 mg/dl was observed in two patients. Four patients developed end-stage renal disease during the follow-up period; three were currently treated with hemodialysis and one with cadaveric kidney transplantation. Clinical and important laboratory data at presentation are summarized in Table 1.

An immunohistochemical study of available kidney specimens was performed in five samples from previously diagnosed AS patient. Immunohistochemical studies of skin biopsied samples were performed in all eleven AS patients, seven first-degree relative females (BS-II:2, MW-II:4, MW-III:2, PS-I:1, and SW-II: two with microscopic hematuria; RL-II:6 and SM-II:3 with normal urinalysis), and four suspected patients belonging to three additional unrelated families. The mother of patient SW-III: 2 suffered from chronic kidney

disease of which certain diagnosis was not made till the time of skin biopsy. The pedigree of AS patients and relatives is shown in Fig. 1. Clinical and laboratory data of relatives and suspected individuals at the time of presentation are summarized in Table 2.

Expression of ·[IV] collagen chains in normal tissue

The results of indirect immunofluorescent studies of α[IV] collagen chains in normal kidney are shown in Fig. 2 and Fig. 3 (upper row). In accordance with the previous studies, α1[IV] staining was consistently observed within all renal basement membrane including the GBM, glomerular mesangium, Bowman's capsular basement membrane (BCBM) and the TBM. The α3[IV] chain was mainly distributed in the GBM, and focally in the TBM and BCBM. A diffuse pattern of α5[IV] chain was restricted to the GBM and BCBM, and in part of the TBM, but not in the mesangium.

Table 1. Clinical and initial laboratory data at presentation of 11 AS patients included in the study

Patient	Age at presentation	Family history	Eye abnormalities	Sensorineural hearing loss (audiometry)	Urinalysis		Urine protein (gram/d)	Creatinine clearance (ml/min)	Serum BUN/Cr (mg/dl)
					Hematuria	Albumin			
BS-III:1	7	+	-	-	gross	4+	0.5	90.3	16/0.5
JR-II:5	16	+	-	+	micro	2+	0.6	17.0	16/2.2
MW-III:1	9	+	-	+	gross	1+	0.6	153.5	10/0.4
PC-II:3	27	+	-	-	micro	1+	1.9	77.8	15/1
PC-III:2	3	+	-	NA	gross	1+	-	-	15/0.6
PS-II:1	3	+	-	-	gross	neg	0.1	55.4	17/0.4
PY-II:7	18	+	+	+	micro	trace	2.1	32.5	15/1.5
RL-III:1	5	-	-	+	gross	4+	1.7	55.3	15/0.5
SM-III:2	11	-	-	-	gross	1+	-	-	14/0.8
SW-III:2	3	+	-	-	gross	trace	-	-	16/0.3
YK-II:1	14	-	-	+	gross	neg	0.1	125.5	16/0.7

Abbreviations: AS: Alport's syndrome, BUN: blood urea nitrogen, Cr: serum creatinine, NA: not available

Table 2. Clinical and initial laboratory data of 7 first degree relatives of AS patients (all females) and 4 suspected individuals with family history of renal disease included in the study

Patient	Sex, Age	Hypertension	Eye abnormalities	Sensorineural hearing loss (audiometry)	Urinalysis		Urine protein (gram/d)	Creatinine clearance (ml/min)	Serum BUN/Cr (mg/dl)
					RBC/HPF	Albumin			
First degree relatives									
BS-II:2	F, 38	-	-	-	20-30	neg	0.6	113.1	12/0.7
MW-II:4	F, 30	-	-	-	10-15	3+	0.7	91.7	11/1
MW-III:2	F, 8	-	-	-	5-10	neg	0.1	110.9	13/0.6
PS-I:1	F, 43	-	-	-	5-10	trace	0.5	69.1	12/0.7
RL-II:6	F, 44	-	-	-	0-1	neg	0.1	121.0	-/0.7
SM-II:3	F, 52	+	-	+	0-1	neg	0.1	NA	18/1.2
SW-II:2	F, 34	+	-	-	10-20	2+	0.3	6.62	56/6.2
Suspected AS individuals									
BJ-1	M, 15	-	-	+	20-30	2+	5.6	30.7	28/2.8
BJ-2	F, 12	-	-	-	50-100	neg	0.4	NA	8/0.4
JP	M, 15	-	-	+	50-100	3+	10.6	NA	38/5.5
OS	F, 33	-	-	-	10-20	1+	NA	NA	10/1.0

Abbreviation: AS: Alport's syndrome, BUN: blood urea nitrogen, Cr: serum creatinine, F: female, HPF: high power field, M: male, NA: not available, RBC: red blood cell

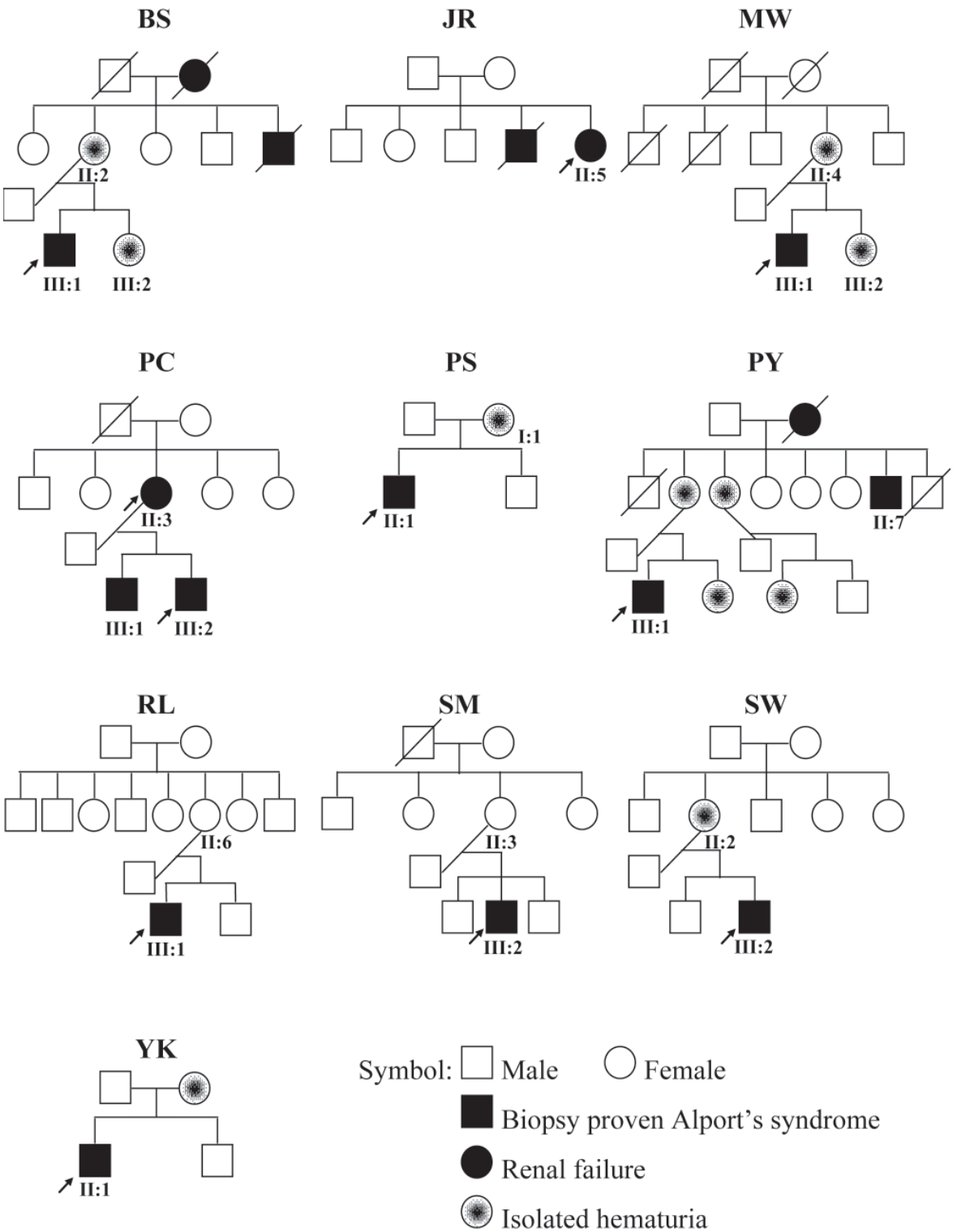

Fig. 1 Pedigrees of 10 families with Alport's syndrome included in the study

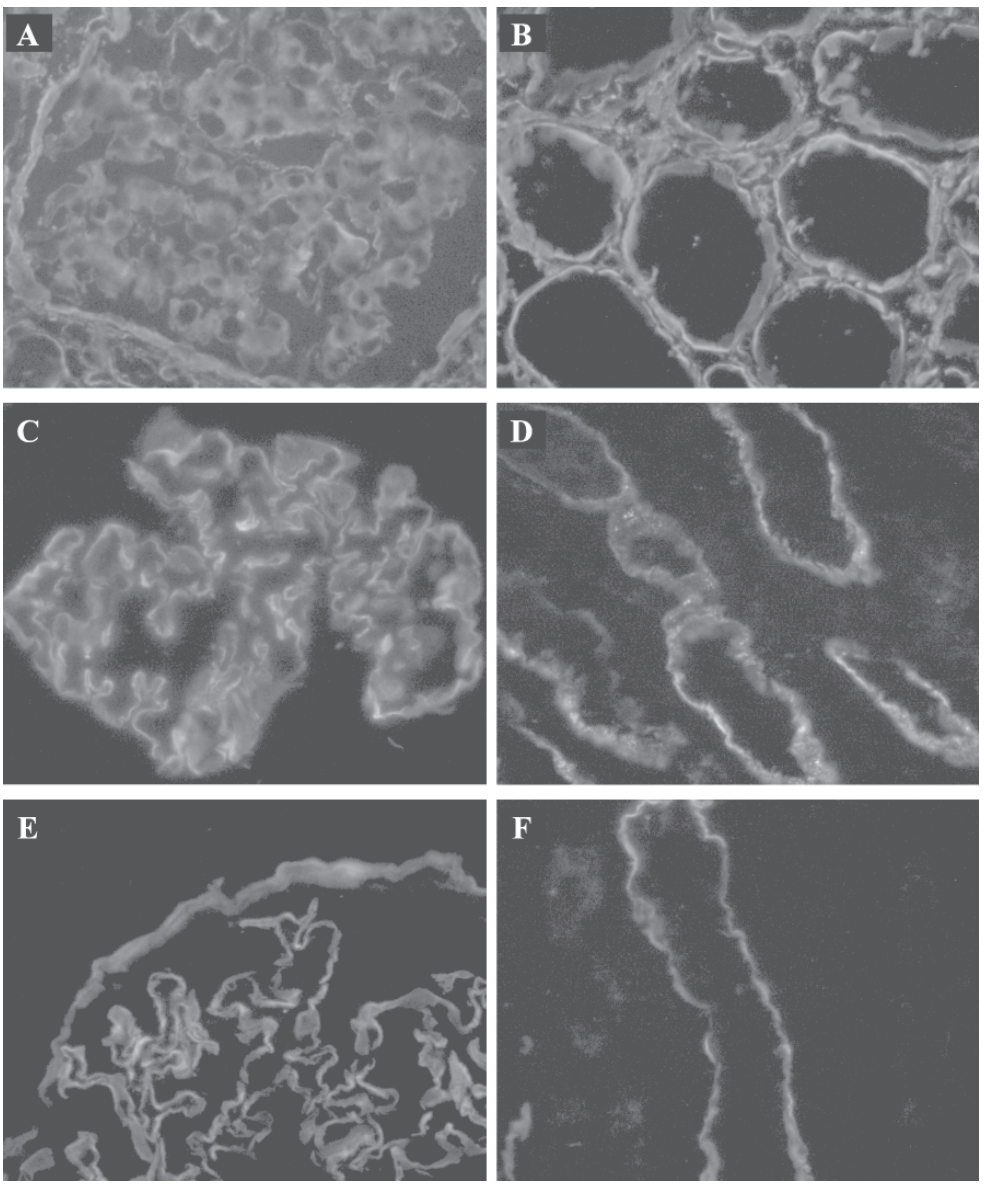

Fig. 2 Distribution of type IV collagen α chains in normal human kidney as shown by indirect immunofluorescence studies using specific monoclonal antibodies: A and B, α1[IV] in glomerulus and tubule; C and D, α3[IV] in glomerulus and tubule; E and F, α5[IV] in glomerulus and tubule. See detail in text

The results of indirect immunofluorescent studies of α[IV] collagen chains in the skin are shown in Fig. 4 (upper row). In all normal controls, staining of α1[IV] was positive in the EBM and blood vessels of subcutaneous tissues while staining for α5[IV] was found in a continuous uninterrupted pattern in the EBM. Similar to the previous studies, staining with antibodies to α3[IV] collagen chains (MAB3) in the skin tissue was constantly negative (not shown).

Expression of α[IV] collagen chains in AS patients

The expression of α1, α3, and α5[IV] collagen chains in the kidney was investigated in the available biopsied tissue samples of five definite AS patients: BS-III:1, MW-III: 1, PS-II: 1, SW-III: 2 and YK-II: 1, all were male. Fig. 3 shows the representative findings of abnormal staining pattern for α[IV] in the glomerulus of previously diagnosed male patients (middle row) compared to normal control (upper row).

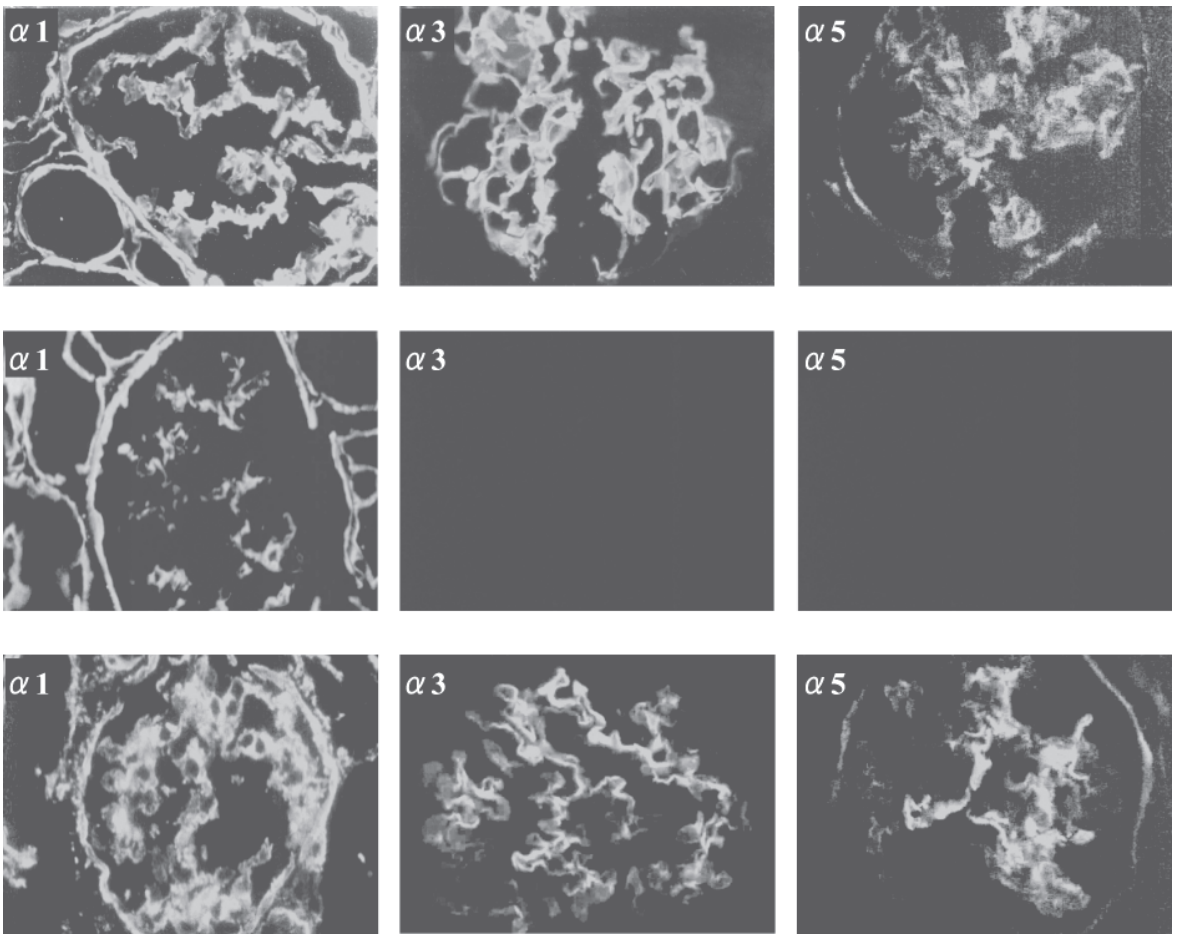

Fig. 3 Immunofluorescence staining for collagen type IV chains of renal specimens from normal control (first row), AS patients (second row), and female relatives (third row). Note normal distribution of α1[IV] in all samples, complete absence of the α3-α5[IV] network in the AS males, and segment reduction in the heterozygous females

Staining for α1[IV] within all renal basement membrane is similar to the controls. All patients in this group, except BS-III:1, showed a complete absence of α3[IV] and α5[IV] staining in the GBM, BCBM and TBM. Staining for α3 and α5[IV] chains in the renal tissue from BS-III:1 was observed in a continuous pattern in both the GBM and TBM (not shown).

Fig. 4 shows the typical findings of α[IV] collagen chains in the skin tissue of previously diagnosed AS patients (middle and lower rows) compared to normal (upper row). Normal staining with MAB1 was observed within the EBM in all cases. All nine male patients showed negative staining or very slight reactivity with MAB5 antibodies on the skin tissue indicating that the α5[IV] chain is absent in the EBM. One female patient (PC-II:3) exhibited a discontinuous or mosaic immunofluorescence pattern with MAB5 while the other (JR-II:5) exhibited a normal linear staining (not shown).

Expression of α[IV] collagen chains in AS relatives and suspected individuals

Sections of skin biopsy specimens of five female relatives with hematuria and two relatives with normal urinalysis were stained for α1 and α5[IV] collagen chains. A normal continuous staining pattern was observed in the tissue from both individuals with normal urinalysis (RL-II: 6 and SM-II: 3) while all samples from those with hematuria exhibited a discontinuous or mosaic pattern of anti α5[IV] staining. Renal

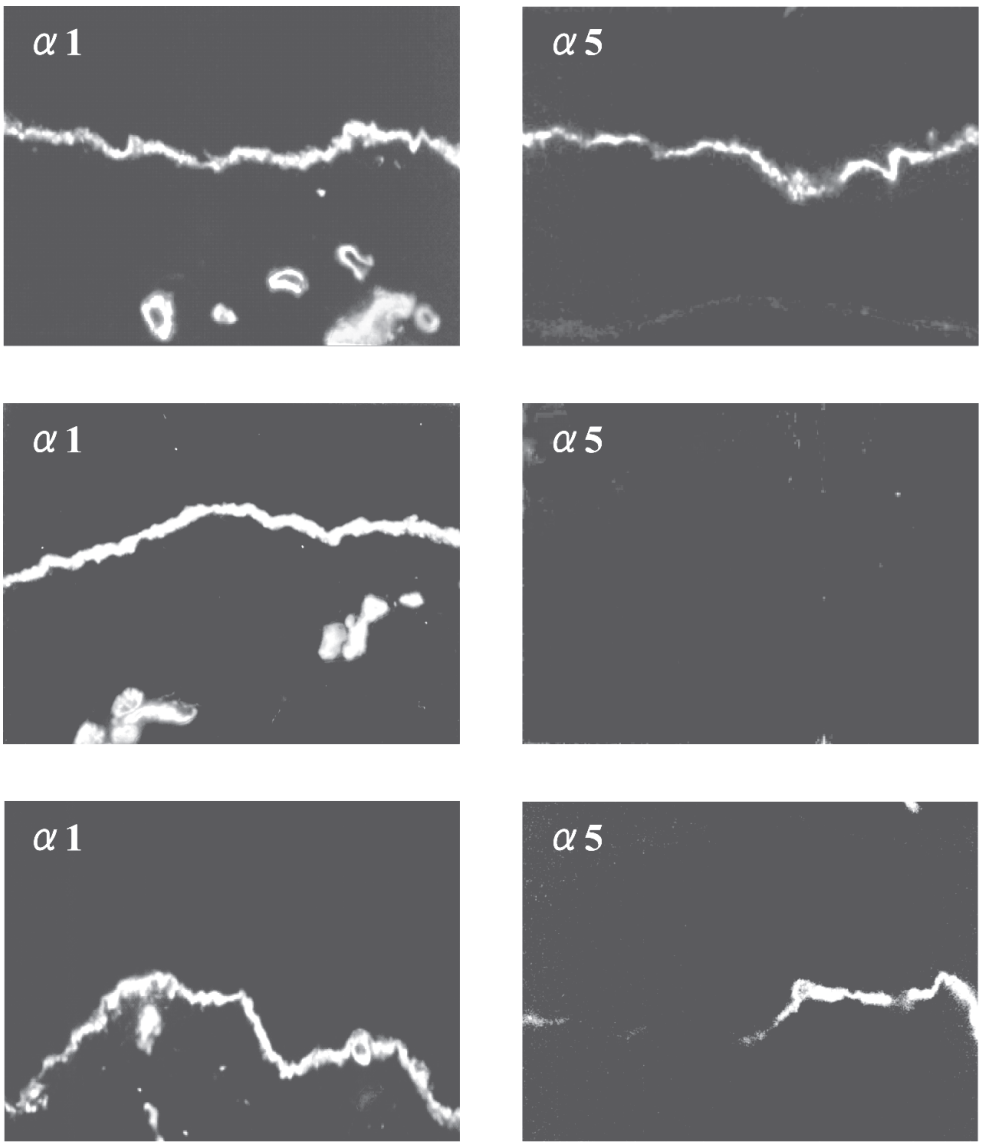

Fig. 4 Immunofluorescence staining for collagen type IV chains of skin specimens from normal control (first row), male AS patients (second row), and female (third row). Note normal distribution of α1[IV] in all samples, and the linear staining of α5[IV] in the epidermal basement membrane of control, complete absence in the AS males, and interrupted/mosaic pattern in the heterozygous females

biopsy was subsequently performed to confirm the diagnosis in two individuals (BS-II:2 and MW-III:2). However, typical GBM changes were not observed by electron microscopic examination, the diagnosis of AS can be made by demonstration of a discontinuous immuno-fluorescence pattern with anti α3 and α5[IV] antibodies in the GBM, BCBM, and TBM (Fig. 3, lower row).

We also studied the expression of the α5[IV] collagen chain in the skin tissue of four suspected individuals with hematuria and a family history of kidney disease. Both males (BJ-1 and JP) exhibited negative reactivity with anti α5[IV] in the EBM and both females (BJ-2 and OS) showed a discontinuous staining pattern. As we observed 100% specificity of these abnormal staining patterns in the skin tissue (similar

to all previous reports), the diagnosis of AS was made in all four patients.

Discussion

The α3, α4, and α5 chains of type IV collagen form a network that is the major collagenous component of the GBM and certain other basement membranes including lung, testis, the lens capsule, and the Organ of Corti[2,13]. Many observations suggest that AS-related mutation affecting any of these type IV collagen chains disrupt the formation of the putative α3-α4-α5[IV] network leading to abnormal expression of all protomers. The mechanism responsible for this pheno-menon has been investigated. In regards to XL-AS, Reeders has proposed three potential explanations: 1) α3[IV] and α4[IV] are synthesized normally, but the incorporation of these chains into the GBM is impaired by the mutation in α5[IV]; 2) normal transcription and/or translation of *COL4A3* and *COL4A4* is prohibited by the α5[IV] mutation; 3) α3[IV] and α4[IV] chains are normally synthesized and incorporated into the GBM, but immunoreactivity is blocked by the mutation in α5[IV][14]. Heidet et al have shown that in patients with XL-AS, both *COL4A3* and *COL4A4* genes are actively transcribed in the podocytes, but the absence or the synthesis of an abnormal α5[IV] chain can prevent the integration of the α3[IV] to α4[IV] chains[15]. As a result, type IV collagen α chains that cannot assemble into a network are prone to a rapid degradation. The absence of α[IV] collagen chains from the GBM has not been reported in any disease other than AS, making this a specific finding on renal tissue examination in addition to conventional electron microscopic studies. The reliability of abnormally distributed or even an absent α3-α4-α5[IV] network has been estimated in about 80% of males and 60-70% of females with XL-AS, as well as patients with AR-AS[6,7,11].

In the other basement membranes, the α5 and α6 chains of type IV collagen form a distinct network of α5-α5-α6[IV] as a constituent of Bowman's capsules, TBM and EBM. This α5-α5-α6[IV] network is also disrupted in XL-AS, thus, immunohistochemical study of the skin has been considered an option in evaluating a patient who is suspected of having XL-AS. Most published series reported the absence of α5[IV] in the EBM in nearly 80% of male patient (hemizygote) or a discontinuous/mosaic pattern in female (heterozygote) with XL-AS, whereas uninterrupted linear staining was consistently observed in normal control and patients with AR-AS[10,16,17].

In this study, the immunohistochemical distribution of type IV collagen chains has been investigated with a set of monoclonal antibodies recognizing the α1, α3 and α5 chains of type IV collagen in the renal and skin tissue of previously diagnosed XL-AS patients, their relatives and suspected individuals. Four of five male patients with available kidney specimen for examination as well as two female relatives with micro-hematuria showed an abnormal staining pattern for α3 and α5[IV] collagen chains in the GBM. This finding is specific for AS since all control samples showed a continuous and uninterrupted staining pattern for both α3 and α5[IV]. Thus, studies in the renal tissue reveal a specificity of 100% and sensitivity of 85.7%. In the case of BS-III:1, a normal uninterrupted pattern of α3 and α5[IV] staining was observed in the GBM whereas α5[IV] staining was negative in the EBM. Thus, it is likely that this *COL4A5* mutation has different effects in the COL4A3-COL4A4-COL4A5 network of the GBM compared with the COL4A5-COL4A6 network of the EBM, as proposed by Naito et al[18]. It is interesting to note that in the case of BS-III:2 and MW-III:2 (both females), changes in the GBM were not characteristic but certain diagnosis can be made by demonstration of an overall decrease in α3 and α5[IV] staining intensity with segmental reduction in the capillary loops. This is the added advantage of using immunohistochemical study to support the diagnosis of AS, in particular of X-linked heterozygotes with minor symptoms.

Analysis of the skin tissue immunohistochemistry in this study can identify AS by demonstration of negative or very slightly positive staining for α5[IV] in the EBM in all eleven males (9 patients and 2 suspected individuals), while a discontinuous pattern was observed in eight of nine females (1 of 2 patients, 5 relatives with hematuria and 2 suspected individuals). All normal controls and two relatives with normal urinalysis showed positive continuous staining of α5[IV] in the EBM. Thus, the sensitivity and specificity of skin tissue immunohistochemistry to diagnose AS are 91% and 100% respectively, and the findings indicate that most mutant COL4A5 in Thai patients commonly lead to misincorporation of the COL4A5-COL4A6 network.

Normal expression of α5[IV] in the EBM of one definite AS female (JR kindred) can be explained in that she might have had AR-AS, or 'minor' *COL4A5* mutation that does not prevent incorporation in the COL4A5-COL4A6 network, or the result of random inactivation of the X chromosome in which a high pro-

portion of cells carry a normal, activated chromosome. Definite diagnosis might require further study of α3 and α5[IV] expression in the renal tissue and/or mutation analysis of the type IV collagen genes. Recently, the use of confocal laser scanning microscopy (CLSM) has been proven to improve the spatial resolution of the α5[IV] chain distribution in the EBM[19]. In our opinion, skin immunohistochemical study under conventional examination, and if needed, with CLSM, is able to detect most cases of XL-AS, thus allowing delay or avoidance of more invasive and costly procedures like renal biopsy and genetic investigation, which has been shown to have a detection limit in nearly 80% of cases[20].

In summary, the combination of clinical data and immunohistochemical study of skin biopsied tissue can identify a large proportion of AS patients in our country, with a specificity of 100% and sensitivity of 95%. Therefore, immunohistochemical study of the skin has a diagnostic utility and should be considered as the first step in screening individuals suspected of AS. In addition, when the kidney biopsy is performed, immunohistochemical studies of α[IV] collagen chains are required to support the diagnosis of AS, especially in the case of minor symptoms and ultrastructural changes.

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การวินิจฉัยโรคอัลพอร์ทโดยการตรวจทางอิมมูโนฮิสโตเคมิสตรีย์

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คำนำ: *โรคอัลพอร์ทเป็นโรคไตอักเสบทางพันธุกรรมที่เกิดจากความผิดปกติในการสร้างคอลลาเจนประเภทสี่ ผู้ป่วยจะมีอาการและอาการแสดงตั้งแต่ปัสสาวะมีเม็ดเลือดแดงมากผิดปกติ โปรตีนรั่วในปัสสาวะ หน้าที่ไตบกพร่อง และอาจพบร่วมกับความผิดปกติของตาและการได้ยิน ในอดีต การวินิจฉัยโรคนี้ต้องอาศัยการตรวจชิ้นเนื้อไตทางกล้องจุลทรรศน์อิเล็กตรอนเท่านั้น ซึ่งในบางครั้งไม่สามารถให้การวินิจฉัยโรคที่จำเพาะได้*

วัตถุประสงค์: *เพื่อนำเทคนิคทางอิมมูโนฮิสโตเคมิสตรีย์มาตรวจสอบการแสดงออกของคอลลาเจนประเภทที่สี่ ชนิดต่าง ๆ ในเนื้อเยื่อ ผิวหนังและเนื้อไต มาใช้ร่วมในการวินิจฉัยโรค ซึ่งยังไม่เคยมีการนำเทคนิคนี้มาใช้ในประเทศไทยมาก่อน*

วัสดุและวิธีการ: *กลุ่มตัวอย่างคือ ผู้ป่วยที่ได้รับการวินิจฉัยจากการตรวจชิ้นเนื้อไตในช่วงปี พ.ศ. 2528 ถึง พ.ศ. 2548 จำนวน 11 ราย รวมทั้งญาติผู้ป่วย 7 ราย และผู้ที่สงสัยว่าเป็นโรคนี้แต่ไม่ได้ตัดชิ้นเนื้อไตอีก 4 ราย โดยมีชิ้นเนื้อผิวหนังคนปรกติและชิ้นเนื้อไตของผู้ที่ไม่ใช่อัลพอร์ทอย่างละ 8 รายเป็นกลุ่มควบคุม*

ผลการศึกษา: *เมื่อนำชิ้นเนื้อไตของผู้ป่วยจำนวน 5 ราย มาทำการตรวจย้อมเพิ่มเติมด้วยแอนติบอดีต่อคอลลาเจนประเภทสี่ ชนิดอัลฟาสาม และอัลฟาห้า แล้วพบว่า ไม่ติดสีทั้งสิ้น 4 ราย ติดสีปรกติ 1 ราย ส่วนการตรวจชิ้นเนื้อผิวหนังของผู้ป่วยเมื่อย้อมด้วยแอนติบอดีต่อคอลลาเจนประเภทสี่ ชนิดอัลฟาห้า พบว่าให้ผลลบจนถึงติดสีจางกว่าคนปรกติจำนวน 9 ราย ติดสีเหมือนคนปรกติจำนวน 1 รายและติดสีไม่สม่ำเสมอจำนวน 1 ราย ส่วนในกลุ่มควบคุมให้ผลการตรวจเป็นปรกติทั้งหมด นอกจากนั้น เมื่อตรวจชิ้นเนื้อผิวหนังของญาติผู้ป่วยอัลพอร์ท (มารดาหรือน้องสาว) ที่มีเม็ดเลือดแดงในปัสสาวะจำนวนทั้งสิ้น 5 ราย พบว่าติดสีไม่สม่ำเสมอทั้งหมด ส่วนการตรวจในผู้ที่สงสัยว่าเป็นอัลพอร์ทพบมีความผิดปกติชนิดไม่ติดสีในผู้ป่วยชาย 2 ราย และติดสีไม่สม่ำเสมอในผู้ป่วยหญิง 2 ราย*

สรุป: *เทคนิคทางอิมมูโนฮิสโตเคมิสตรีย์เพื่อตรวจหาการแสดงออกของคอลลาเจนประเภทสี่ ชนิดอัลฟาสาม และอัลฟาห้า ในชิ้นเนื้อผิวหนัง สามารถนำมาใช้ในการวินิจฉัยโรคอัลพอร์ทได้ โดยมีความไวและความจำเพาะในการตรวจเท่ากับร้อยละ 91 และ 100 ตามลำดับ จึงควรนำมาใช้ในการตรวจคัดกรองผู้ป่วยโรคอัลพอร์ทในประเทศไทย นอกเหนือไปจากการตรวจชิ้นเนื้อไตที่ทำอยู่ในปัจจุบัน*