Kidney Medicine

Glomerular Basement Membrane Protein Expression and the Diagnosis and Prognosis of Autosomal Dominant Alport Syndrome

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Alport syndrome is a hereditary glomerular nephritis associated with hearing loss and eye abnormalities and is classified as X-linked Alport syndrome, autosomal recessive Alport syndrome, and autosomal dominant Alport syndrome. Autosomal dominant Alport syndrome is caused by a mutation in the gene encoding type IV collagen α3 (α3[IV]); (COL4A3), or α4 (α4[IV]); (COL4A4). Autosomal dominant Alport syndrome progresses more gradually than male X-linked Alport syndrome and autosomal recessive Alport syndrome. Differentiating autosomal dominant Alport syndrome from thin basement membrane nephropathy, which shows better kidney prognosis, remains challenging. Because autosomal dominant Alport syndrome is linked to a heterozygous mutation, type IV collagen is produced by the wild-type allele, and all α(IV) chains are supposed to be normally expressed. In this study, the pathologic findings of a patient with Alport syndrome with a novel COL4A4 heterozygous nonsense mutation were investigated. We observed weaker staining of α5(IV) in the glomerular basement membrane and enhanced expressions of α2(IV), laminin, and fibronectin, which were assumed to be caused by compensatory mechanisms for lack of enough α3α4α5(IV) expression in the glomerular basement membrane. These findings may be useful not only for differentially diagnosing autosomal dominant Alport syndrome from thin basement membrane nephropathy, but also for determining the extent of progression and predicting kidney prognosis.

INTRODUCTION

Alport syndrome is a hereditary glomerular nephritis associated with hearing loss and eye abnormalities and is classified as X-linked Alport syndrome, autosomal recessive Alport syndrome, or autosomal dominant Alport syndrome.[1] X-linked Alport syndrome is caused by a mutation in type IV collagen α5 chain (α5(IV)) gene. Autosomal recessive Alport syndrome and autosomal dominant Alport syndrome originate because of different mutations in the type IV collagen α3 (α3[IV]) gene (COL4A3) or α4 (α4[IV]) gene (COL4A4). Autosomal dominant Alport syndrome progresses more gradually than what is observed in the cases of X-linked Alport syndrome in males and autosomal recessive Alport syndrome.

Notably, differentiating autosomal dominant Alport syndrome from thin basement membrane nephropathy, which shows better kidney prognosis, remains challenging. Pathologically, Alport syndrome shows thickening and lamellation of the glomerular basement membrane (GBM); conversely, thin basement membrane nephropathy shows only GBM thinning. In heterozygous COL4A3 knockout mice, only GBM thinning initially occurs, as observed in thin basement membrane nephropathy. However, GBM thickening and lamellation, as seen in Alport syndrome, manifest with aging.[2] Heterozygous mutations in COL4A3 or COL4A4 were detected in ∼40% of thin basement membrane nephropathy cases.[3] Several patients who were diagnosed with thin basement membrane nephropathy later developed kidney failure.[4] Collectively, among patients initially diagnosed with thin basement membrane nephropathy, there may be many more patients with autosomal dominant Alport syndrome. Thus, a recent report has advocated for classifying Alport syndrome as X-linked, autosomal, and digenic without the name of thin basement membrane nephropathy.[5]

In GBM, the α3(IV), α4(IV), and α5(IV) chains create trimers that form collagen fibers.[1] In male X-linked Alport syndrome and autosomal recessive Alport syndrome, production of 1 α chain is insufficient, while no α3(IV), α4(IV), or α5(IV) chains are expressed in the GBM. However, in female X-linked Alport syndrome, when a normal α5(IV) chain is present, 1 allele is inactivated in the X chromosome and expressed as a mosaic α5(IV).[6] Because autosomal dominant Alport syndrome is a heterozygous mutation, type IV collagen is produced from the normal allele, and all α(IV) chains are normally expressed.[7,8]

Expression of type IV collagen α1 (α1[IV]) and α2(α2[IV]) was observed in the GBM of individuals with Alport syndrome and in COL4A4 knockout mice.[9-11] Additionally, increased ectopic expression of extracellular matrix, such as laminin and fibronectin, was observed in the GBM of patients with Alport syndrome and COL4A3 knockout mice.[4,12,13]

The pathologic findings of a patient with Alport syndrome with a novel COL4A4 heterozygous nonsense mutation were investigated. We observed weaker staining of α5(IV) in the GBM and enhanced expressions of α2(IV), laminin, and fibronectin.

CASE REPORT

The patient was a woman in her early 20s whose chief concern was microscopic hematuria. Her maternal grandfather was undergoing hemodialysis and her mother and younger brother had microscopic hematuria. The patient had had occult blood in her urine every year for the past 19 years. At presentation, urinary protein excretion was 2^+, 0.91 g/g of creatinine; occult blood was 3^+; and urinary sediment erythrocytes were 50 to 99/high-power field (few poikilocytes); thus, the patient was admitted to our department for kidney biopsy. Serum creatinine level was 0.6 mg/dL, and estimated glomerular filtration rate was 101.9 mL/min/1.73 m^2.

Light microscopy revealed that 5 of 30 glomeruli had completely sclerosed, but there were no crescents, adhesion, or focal glomerular sclerosis. Although periodic acid–methenamine silver staining showed no loop duplication or spike formation, there was loop wrinkling, partial hypochromasia, and lamellation (Fig 1). Fluorescent

antibody analysis revealed no immunoglobulin or complement deposition. Electron microscopy findings revealed notable GBM wrinkling and loop lumen collapse and segmented areas of mild thinning of the compact GBM layer, but much of the GBM showed normal thickness. Areas of lamellation and mild reticulation were observed in the compact GBM layer (Fig 1).

In type IV collagen staining of the GBM, double-staining of α2 and α5 indicated weak positivity with partially reduced green α5 staining in the GBM, while α2 expression was stronger in the weakened areas (Fig 2). Furthermore, we observed increased staining of extracellular matrix, laminin, and fibronectin in the GBM in our case as compared to the control (Fig 2).

We obtained the patient's consent to conduct genetic analysis for a definitive diagnosis and conducted target sequencing with a next-generation sequencer. This method used a gene panel targeting hereditary kidney disease as previously described.[14] We detected a
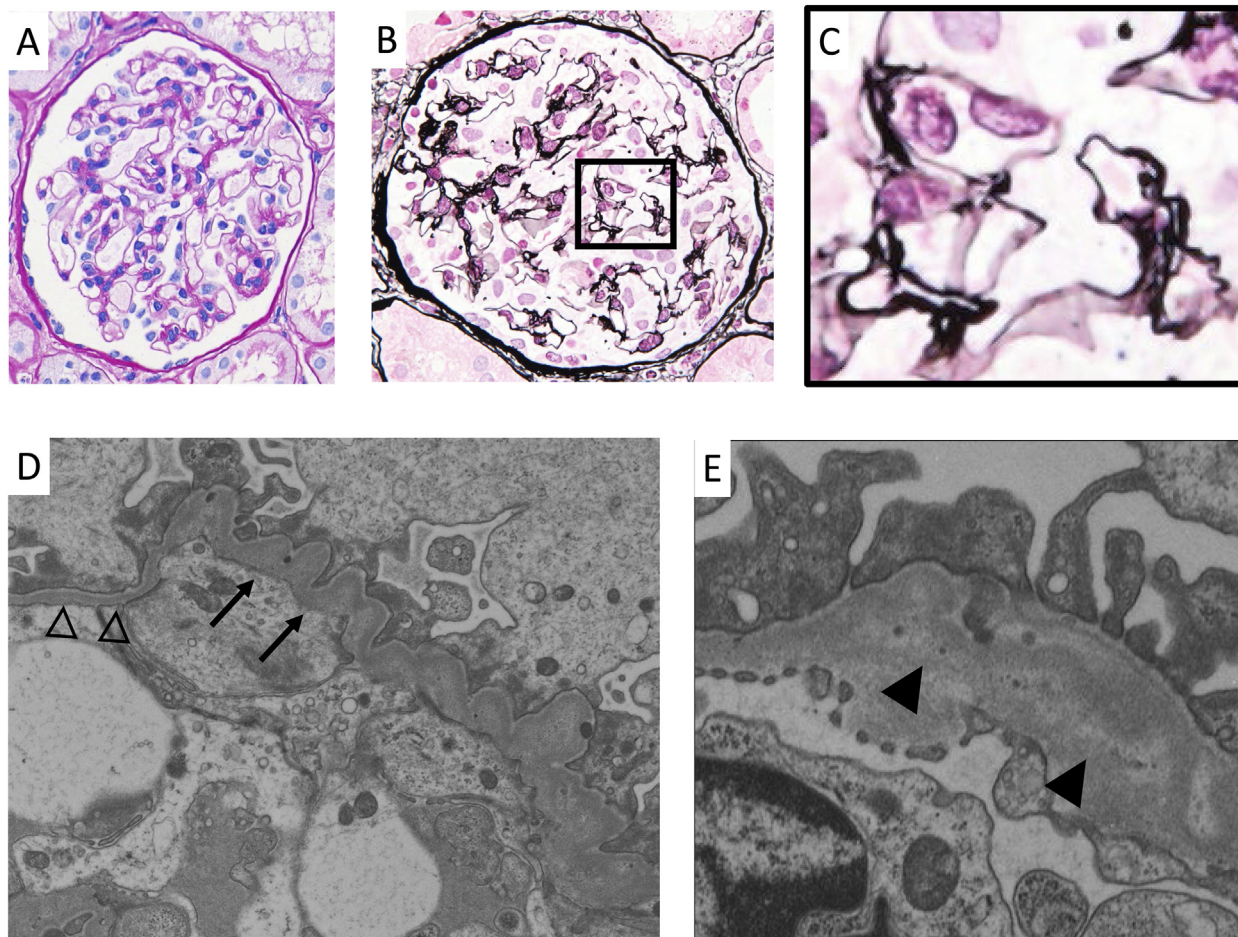

Figure 1. (A-C) Light microscopy and (D, E) electron micrographs of the kidney biopsy specimen from autosomal dominant Alport syndrome. (A) Glomeruli show no proliferative or sclerotic changes (periodic acid–Schiff stain; original magnification, ×200). (B) Segmental irregular thickening of glomerular basement membranes (GBMs). (C) Enlarged figure of square in B. (B, C: periodic acid–methenamine silver stain; original magnification, B: ×200, C: ×400). (D) Irregular distribution of thin GBM segments (arrowheads) with segmental wrinkling (arrows). (E) Segmental lamellation and splitting in GBM (arrowheads) (uranyl acetate-lead citrate; original magnification, D: ×5,000; E: ×10,000).
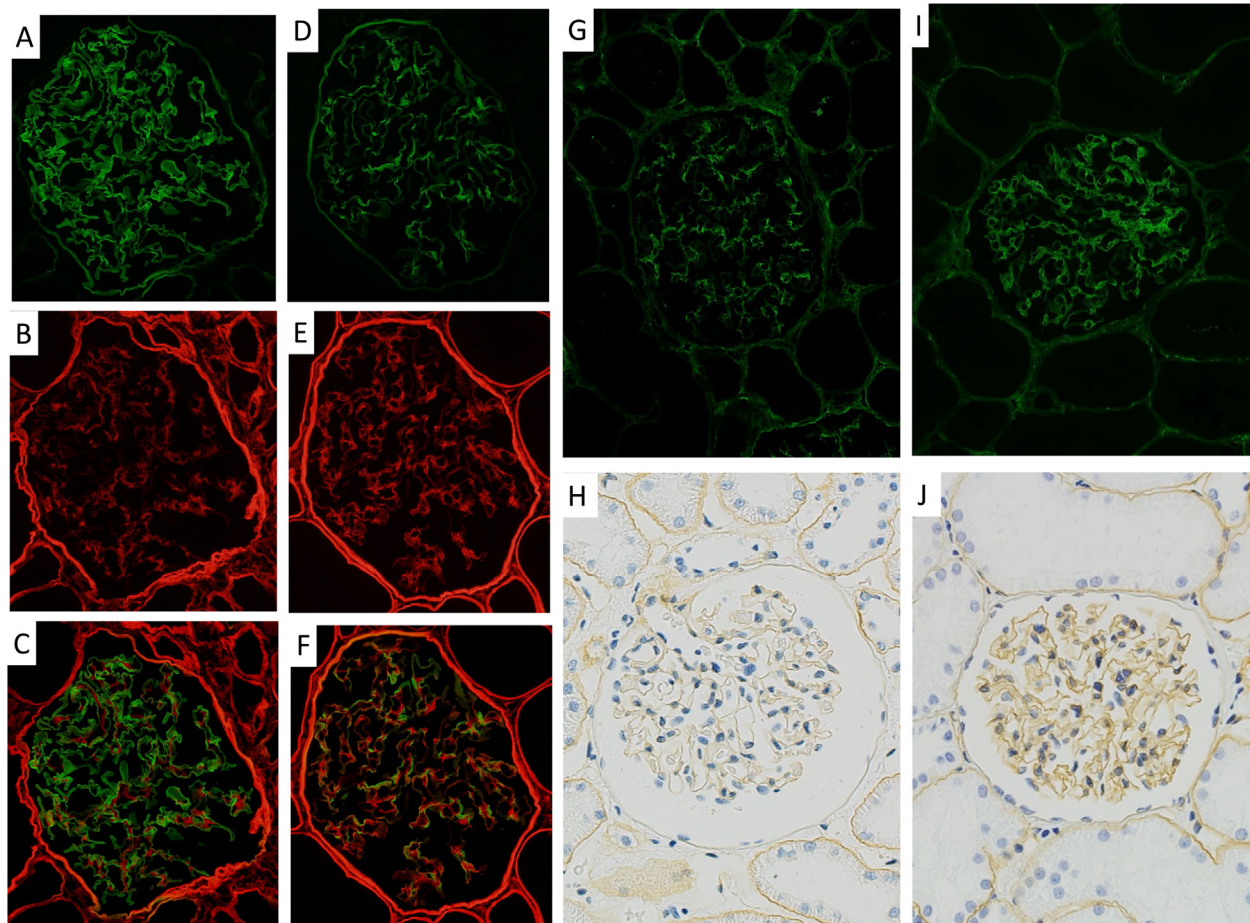
Figure 2. Immunohistochemical staining of type IV collagen, fibronectin, and laminin. Type IV collagen staining findings in (A-C) control and (D-F) autosomal dominant Alport syndrome using antibodies for (A, D) α5(IV) or (B, E) α2(IV) chains. In a control kidney, (A) the α5 chain shows positive staining in the glomerular basement membrane (GBM) and (B) α2 chain shows positive staining in the mesangial matrix. In autosomal dominant Alport syndrome, (D) α5 chain shows weak positivity with partly reduced staining in GBM, while (E) α2 chain shows positive staining in mesangial matrix and segmental upregulation in GBM. (C, F) Double labeling was performed with anti-α2(IV) in red and anti-α5(IV) in green (all: original magnification, ×200). Combination of 2 monoclonal antibodies staining α5(IV) green (fluorescein isothiocyanate; H53 rat immunoglobulin G2 [IgG2]a/κ and B51 rat IgG2a) and α2(IV) red (Texas red; H25 rat IgG1/κ) were used (Shigei Medical Research Institute: CFT-45325). Staining findings for fibronectin and laminin in (G, H) control and (I, J) autosomal dominant Alport syndrome. In the control kidney, (G) fibronectin and (H) laminin showed weakly positive staining in GBM. In autosomal dominant Alport syndrome, both (I) fibronectin and (J) laminin showed increased staining in GBM, compared to control (all: original magnification, ×200). Fluorescein-conjugated goat anti-human fibronectin antibody (#55193; ICN/CAPPEL) and rabbit polyclonal anti-laminin antibody (Z0097; Dako) were used.

heterozygous nonsense mutation (c.C2566T:p.Q856X) in *COL4A4* (NM_000092). This novel gene mutation was located within exon 14 in the collagenous domain.

DISCUSSION

When the glomerulus forms during the fetal stage, the capillary loop initially comprises α1(IV) and α2(IV) produced from mesangial, endothelial, or epithelial cells. As the glomerulus matures, α1(IV) and α2(IV) disappear from the GBM and are replaced with network-forming α3α4α5(IV) chains, produced exclusively by epithelial cells.[1]

In Alport syndrome, α5(IV) chain expression in the GBM is used as a diagnostic tool and a prognostic factor. α5(IV) is not expressed in the GBM in male X-linked Alport syndrome and autosomal recessive Alport syndrome, which is thought to result in poor kidney prognosis.[15] However, in male X-linked Alport syndrome and autosomal recessive Alport syndrome with missense or in-frame deletion/insertion, α3α4α5(IV) trimers are formed, albeit insufficiently, and α5(IV) chain expression is slightly preserved, resulting in a relatively good kidney prognosis.[15,16] In female X-linked Alport syndrome, either the normal or mutated α5(IV) chain is involved in the expression, with the α5 chain expressed as a mosaic, and

thus kidney prognosis is better than that in male X-linked Alport syndrome.[6] Therefore, α5(IV) chain expression is essential for predicting kidney prognosis in Alport syndrome.

Autosomal dominant Alport syndrome has a normal α3(IV) chain or α4(IV) chain, and α3α4α5(IV) trimers are formed in the GBM. Studies have shown that the α5(IV) chain was expressed normally.[7,8] However, in our patient, part of the α5(IV) chain showed decreased expression (Fig 2). Autosomal dominant Alport syndrome cases have generally good kidney prognosis, but several patients show poor kidney prognosis.[17,18] Our patient was still young and thus kidney function had not declined, but sclerotic glomeruli and weakened expression of the α5(IV) chain may suggest her poor kidney prognosis.

In autosomal dominant Alport syndrome, if the α chain is sufficiently expressed from 1 normal allele, GBM homeostasis is maintained, and it is thought that no GBM abnormalities appear. However, GBM abnormalities occur in autosomal dominant Alport syndrome. Even when expression appears normal in standard α5(IV) staining, the amount of α345(IV) trimers may not be enough to maintain the GBM function. Our patient had a nonsense mutation, which causes nonsense-mediated messenger RNA decay in part, leading to a lack of collagen synthesis from the mutated allele.[19] Moreover, although truncating protein is produced in part, the NC1 domain is deficient, so that α3α4α5 (IV) trimers cannot be formed. In previous reports, patients with autosomal dominant Alport syndrome had

normal expression of the α5(IV) chain. Most patients with autosomal dominant Alport syndrome have nontruncating mutations, enabling the formation of α345(IV) trimers that are unstable.[7,8] Even in patients with truncating mutations, α5(IV) expression may not have been weakened in the early stage of Alport syndrome. Presumably, the amount of type IV collagen to enable GBM function may have been insufficient in autosomal dominant Alport syndrome, leading to decreased α5(IV) expression.

α2(IV) expression in the GBM was increased in our patient (Fig 2). Although α1(IV) and α2(IV) are expressed by mesangial cells, they are no longer expressed in the GBM in adults.[1] However, α1(IV) and α2(IV) are expressed in the GBM of both patients with Alport syndrome and COL4A4 knockout mice.[9-11] The α1(IV) and α2(IV) chains are assumed to be expressed in a compensatory manner in Alport syndrome with weakened expression of α3α4α5(IV).

Furthermore, laminin and fibronectin expression levels were increased in the GBM in our patient (Fig 2). In Alport syndrome, GBM thickening and lamellation occur, unlike in thin basement membrane nephropathy. The extracellular matrix contributes to these changes in the GBM to replace type IV collagen.[10] Expression of extracellular matrix, such as laminin and fibronectin, is also enhanced in patients with Alport syndrome and COL4A3 knockout mice,[4,12,13] which suggests the presence of a compensatory mechanism, similar to the manner of enhanced α2 expression in the GBM.
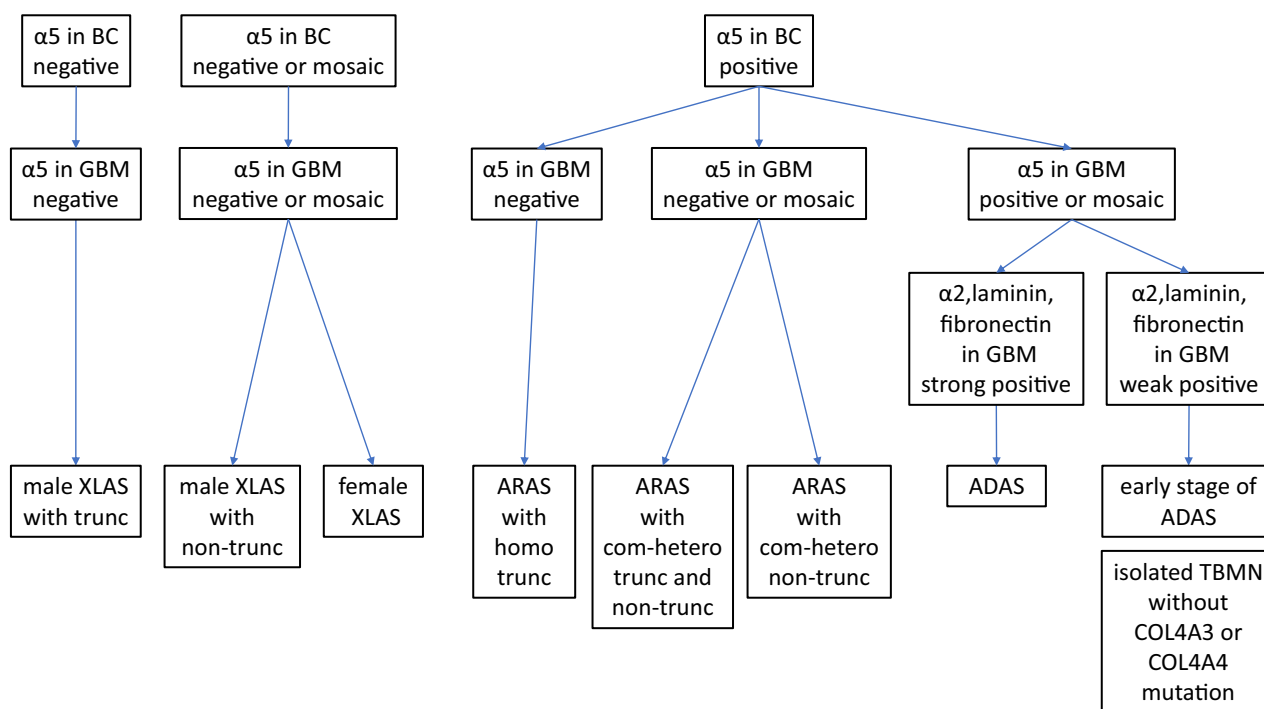

Figure 3. Differential diagnosis in each type of Alport syndrome and isolated thin basement membrane nephropathy. Abbreviations: ADAS, autosomal dominant Alport syndrome; ARAS, autosomal recessive Alport syndrome; BC, Bowman capsule; com-hetero, compound heterozygous; GBM, glomerular basement membrane; homo, homozygous; non-trunc, nontruncating mutation; TBMN, thin basement membrane nephropathy; trunc, truncating mutation; XLAS, X-linked Alport syndrome.

Diffuse thinning of the GBM is reported as the single disease trait in 10% to 20% of Alport syndrome cases, mostly in children.[20] Similar findings were observed in the early stage of COL4A3 knockout mice.[4] Thus, a portion of thin basement membrane nephropathy may merely be the clinical precursor of autosomal dominant Alport syndrome. Reduced collagen expression initially causes GBM thinning like thin basement membrane nephropathy, followed by compensatory increases of extracellular matrix, causing GBM thickening and lamellation. This hypothesis is consistent with a previous report advocating for a new classification of Alport syndrome.[5]

In the new classification, it was recommended that patients should have annual monitoring of blood pressure, urine protein excretion, and kidney function.[5] However, there are no other more concrete proposals to ascertain disease progression. In Alport syndrome lacking normal $\alpha3\alpha4\alpha5(IV)$ chain function, compensatory expressions of extracellular matrix such as $\alpha2(IV)$, laminin, and fibronectin increase to protect the GBM. Expression levels may reflect the progression of Alport syndrome. These findings can be used as criteria to determine the severity of Alport syndrome. A flowchart draft for the differential diagnosis of Alport syndrome and thin basement membrane nephropathy based on type IV collagen, laminin, and fibronectin staining is shown in Figure 3. In autosomal dominant Alport syndrome, there are different phases of the disease in the same patient.

In conclusion, verifying $\alpha5(IV)$ chain expression in the GBM in autosomal dominant Alport syndrome and extracellular matrix expression such as $\alpha2(IV)$, laminin, and fibronectin may enable differential diagnosis of autosomal dominant Alport syndrome and thin basement membrane nephropathy and may determine the extent of pathologic progression, predicting kidney prognosis in Alport syndrome.

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