

Extreme Renal Pathology in Alagille Syndrome



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INTRODUCTION

Alagille syndrome (ALGS) results from mutations in *JAG1* and *NOTCH2* in the Notch signaling pathway.^{1,2} These mutations clinically manifest in various ways, but ALGS is most commonly characterized by a paucity of bile ducts in the liver. ALGS often involves the kidney, which can be characterized by defects in the glomerular vasculature, podocytes, proximal tubules, and renal dysplasia. In addition, altered lipid metabolism in ALGS can cause mesangial lipidosis in the kidney.^{1,3} Few case reports describe the renal manifestations of ALGS.^{1,3–10} Here we report an Alagille syndrome patient with renal dysplasia, renal lipidosis, and bile cast nephropathy. This case highlights the spectrum of renal pathologic findings due to Alagille syndrome that can manifest as a result of defects in the Notch signaling pathway.

CASE PRESENTATION

A 19-year-old male patient with ALGS was treated with a partial external biliary diversion when he was 1 year of age, and did not require liver transplantation. He had Henoch–Schönlein purpura at age 6 years, with acute kidney injury and hypertension that resolved. He also had a history of peripheral pulmonic stenosis, splenomegaly, and nephrolithiasis. He was lost to follow-up from Pediatric Nephrology. His last nephrology visit was 10 years prior to this admission, at which time his plasma creatinine was 0.7 mg/dl, corresponding to an estimated glomerular filtration rate (eGFR) of 68 ml/min/1.73 m². His last visit with Hepatology was 2 years prior to this admission, at which time his laboratory values were as follows: total bilirubin, 18.6 mg/dl; direct bilirubin, 13.8 mg/dl; AST, 146 mg/dl; ALT, 178 mg/dl; alkaline phosphatase, 731 mg/dl; blood urea nitrogen (BUN), 33 mg/dl; and

plasma creatinine, 1.12 mg/dl, corresponding to an eGFR of 56 ml/min/1.73 m².

He presented on this admission with left-sided abdominal pain, gross hematuria, weakness, constipation, and decreased appetite. He had an elevated serum creatinine of 6 mg/dl, BUN of 134 mg/dl, total bilirubin of 33 mg/dl, and direct bilirubin of 26.1 mg/dl. However, his liver enzymes (AST, 102 mg/dl; ALT, 161 mg/dl; alkaline phosphatase, 627 mg/dl) and platelet count ($69 \times 10^3/\mu\text{l}$) were not remarkably different from his usual baseline levels (Table 1). His total cholesterol was unchanged from prior values at 138 mg/dl; however, his high-density lipoprotein (HDL) –cholesterol had decreased from 17 mg/dl to <5 mg/dl. His serum albumin was low at 2.6 mg/dl (previously 3.9 mg/dl 2 years before admission). His urinalysis was notable for numerous red blood cells (not quantified), 3 to 5 white blood cells/hpf, and new onset of proteinuria with a urine protein/creatinine of 0.8 mg/mg. His kidney insufficiency did not improve, and he was started on hemodialysis. A kidney biopsy was performed.

Renal Biopsy Findings

The light microscopic sample contained 11 glomeruli, of which 5 glomeruli were globally sclerosed. The patent glomeruli demonstrated diffuse vacuolization of the glomerular basement membranes and subepithelial “spike” formation highlighted on the methenamine silver stain (Figure 1). Focally, mesangial cells and podocytes showed vacuolization, and some podocytes contained yellow-brown pigment. Interstitial foam cells were not present. The cortex contained patchy and focally marked interstitial fibrosis and tubular atrophy with normally developed tubules. In contrast, some medullary tubules were immature and focally surrounded by spindle cells, which was consistent with

Table 1. Summary of case patient's laboratory results

| Laboratory variable | Current value | Previous value |
|--------------------------|---------------------------|----------------|
| Creatinine | 6 mg/dl | 1.12 mg/dl |
| Serum albumin | 2.6 mg/dl | 3.9 mg/dl |
| Total bilirubin | 33 mg/dl | 18.6 mg/dl |
| Direct bilirubin | 26.1 mg/dl | 13.8 mg/dl |
| AST | 102 mg/dl | Unchanged |
| ALT | 161 mg/dl | Unchanged |
| Alkaline phosphatase | 627 mg/dl | Unchanged |
| Platelets | 69 x 10 ³ /μl | Unchanged |
| Total cholesterol | 138 mg/dl | Unchanged |
| HDL cholesterol | <5 mg/dl | 17 mg/dl |
| Urinalysis | | |
| Red blood cells | Numerous (not quantified) | 0/hpf |
| White blood cells | 3–5/hpf | 0/hpf |
| Urine protein/creatinine | 0.8 g/g | Normal range |

ALT, alanine aminotransferase; AST, aspartate transaminase; HDL, high-density lipoprotein; hpf, high-power field.

renal dysplasia (Figure 2). Some of the distal nephron segments contained yellow–brown casts that were consistent with bile/bilirubin casts (Figure 2), and some tubular epithelial cells contained yellow–brown cytoplasmic inclusions.

Immunofluorescence microscopy (scale, 0–4+) of 3 glomeruli (1 globally sclerotic) showed no staining of the glomeruli, tubular basement membranes, or interstitium for IgG, IgA, IgM, C3, C1q, fibrinogen, κ or λ light chains, or albumin. Tubular casts stained for IgA and κ and λ light chains. The arterioles stained for C3.

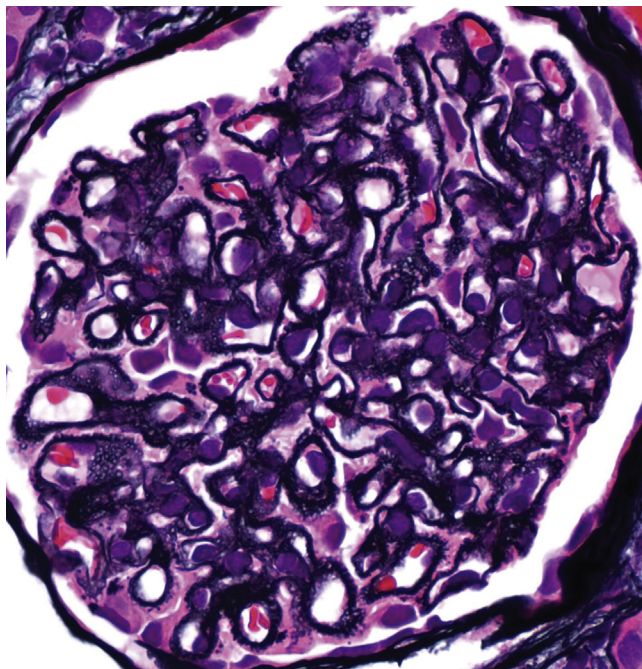


Figure 1. Glomerular basement membranes show a prominent vacuolated appearance and focal subepithelial “spike” formation, which appears identical to membranous nephropathy (Jones methenamine silver, original magnification ×600).

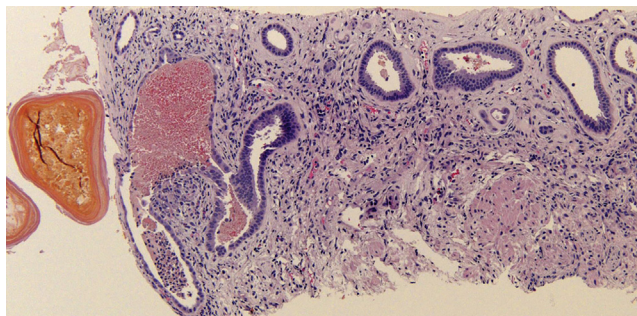


Figure 2. Several immature tubules and a large bile cast is present on the left (hematoxylin and eosin, original magnification ×40).

Ultrastructural evaluation demonstrated diffuse deposition of lamellated electron-dense deposits and vacuoles, which were consistent with lipid droplets, in the glomerular basement membranes (Figure 3). Occasional podocytes and mesangial and endothelial cells contained lipid vacuoles. There were also occasional lipid vacuoles present in the tubular and peritubular capillary basement membranes, interstitium, and smooth muscle cells in arterioles (Figure 4). Immune-type electron-dense deposits were not present. The final diagnosis was basement membrane and mesangial lipidosis consistent with renal involvement by Alagille syndrome, with additional components of bile cast nephropathy and renal dysplasia.

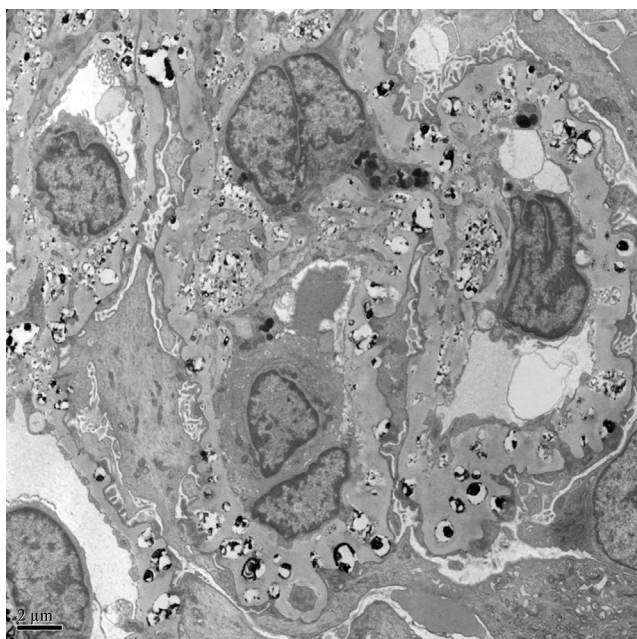


Figure 3. Numerous lipid vacuoles with characteristic electron dense and lucent areas are embedded in the glomerular basement membranes and some mesangial cells. Some of the lipid vacuoles are subepithelial, intramembranous, or subendothelial in location (bar = 2 μm).

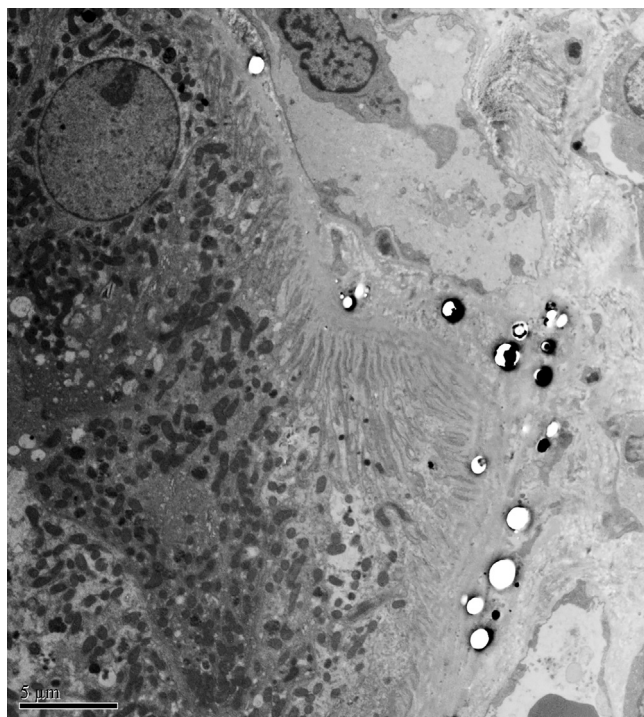


Figure 4. Similar lipid vacuoles also accumulate within the tubular basement membranes (original magnification $\times 5000$).

The patient's kidney function did not improve, and he was discharged on maintenance outpatient hemodialysis.

DISCUSSION

ALGS is an autosomal-dominant inherited disease caused by mutations in either *JAG1* or *NOTCH2* genes involving 94% and 0.8% of probands, respectively.^{1,2} This disorder can affect multiple organs, including the liver, heart, skeleton, eye, face, and kidneys. Clinical penetrance is variable, but most patients present with cholestasis caused by a paucity of intralobular bile ducts.³ Pruritis, xanthomas, and failure to thrive are also common and result from the bile duct dysplasia in the liver.¹ Cholestasis can progress to end-stage liver disease, and liver transplantation is required in 20% to 50% of cases.⁴ Congenital cardiac involvement is common, usually involving the pulmonary arteries and the pulmonary valves, and tetralogy of Fallot is often present.¹¹ The most common skeletal abnormality is a vertebral sagittal cleft, called the butterfly vertebrae. Posterior embryotoxon (prominence of Schwalbe ring) can occur in the eye. ALGS patients can have a characteristic facies: triangular face shape with a prominent forehead, hypertelorism, pointed nose, and saddle nose with bulbous tip.¹ Clinical diagnosis is based on the absence of bile ducts in association with at least 3 additional clinical features among those listed above. Renal and vascular abnormalities are present in 40% to

70% and 10% to 15% of patients, respectively.^{1,5} Clinical renal findings reported include horseshoe kidney, congenital single kidney, a duplex collecting system, renal dysplasia (with or without cysts), renal tubular acidosis, ureteropelvic or vesicoureteral urinary obstruction, vesicoureteral reflex, and transient azotemia.^{1,7,8} These renal abnormalities can cause secondary clinical findings, including metabolic bone disease caused by renal tubular acidosis.¹²

More than 430 mutations in *JAG1* have been identified, and there are no mutational hotspots. These mutations often result in a truncated protein that leads to haploinsufficiency.⁵ *JAG1* is expressed in many adult tissues, including heart, skeletal muscle, kidney, and bone marrow, and in fetal heart, liver, lung, and kidney.¹¹ The expression of *notch2* and *jagged1* is complex and varies during the different stages of kidney development. In the kidney, the ureteric bud arises from the Wolffian duct, invades the adjacent metanephric blastema, undergoes repeated branching, and eventually forms the collecting ducts, calyces, pelvis, and ureter. The ureteric branch tips then induce mesenchymal cells to form nephrogenic precursors including the cap mesenchyme. *Notch2* is expressed in the ureteric bud and cap mesenchyme and immature proximal tubules, and *notch2* and *jagged1* are both expressed in the subsequent epithelial vesicle, comma-shaped body, and S-shaped body. In the mature nephron, glomerular and tubular epithelial cells express *notch2*, whereas *jagged1* is expressed in glomerular endothelial cells and collecting ducts. In mice, this variable expression results in glomerular, podocyte, and proximal tubule defects in animals with *JAG1* and *NOTCH2* mutations.^{5,13} Homozygous *NOTCH2* mutations and homozygous *JAG1* null mutations are embryonically lethal in mice due to vascular defects in the metanephric kidney. Mice heterozygous for mutations in both *JAG1* and *NOTCH2* serve as a model for ALGS, and in these mice, the early stages of kidney development occur normally, but podocytes and proximal tubules do not form, and the glomerulus has vascularization defects.^{5,14,15}

The role of *JAG1* and *NOTCH2* in kidney development likely leads to the clinical and pathologic renal manifestations in ALGS that have been described.^{1,3–10} The defects in proximal nephron development due to *JAG1* or *NOTCH2* may account for renal dysplasia,¹ as seen in our patient. Renal dysplasia is present in most ALGS patients, which is defined by the presence of immature tubules surrounded by collarettes of condensed mesenchyme with or without immature cartilage or cysts on imaging (increased echogenicity with or without renal cysts and small size).^{1,16,17} Renal tubular acidosis, ureteropelvic or vesicoureteral

urinary obstruction, and vesicoureteral reflux can also occur.¹ Chronic kidney disease is common in ALGS, with 1 study reporting a calculated glomerular filtration rate (GFR) of <90 ml/min in 18% of ALGS patients, which did not improve after liver transplantation.⁴ Multiple factors likely contribute to the progressive renal dysfunction that occurs even after liver transplantation, including the effects of long-term immunosuppression.

In addition to renal dysplasia, our patient had bile cast nephropathy, which occurs in patients with severe liver dysfunction. High levels of serum total bilirubin and bile acids result in direct tubular injury, and formation of bile casts leads to nephron obstruction. It can occur in a wide spectrum of severe liver dysfunction, including cholestatic/obstructive jaundice,¹⁸ and to our knowledge this is the first report in the setting of ALGS.

There are only a few reports of ALGS, but all describe glomerular lipid deposition. Alagille *et al.* described mesangiolipidosis in 17 of 23 studied patients, and electron microscopy demonstrated lipid vacuoles in the mesangial matrix.¹ Another study reported similar pathologic findings in 18 of 26 patients, and found that the extent of mesangiolipidosis was related to the degree of cholestasis, with more prominent findings in patients with high bilirubin, high total cholesterol, and high triglyceride levels.⁷ Although the level of cholestasis and hyperlipidemia in our patient was relatively lower than in prior reports, the patient is significantly older than those in the other reports, and the long-term cumulative impact of liver dysfunction could have resulted in the current biopsy findings. Chung-Park *et al.* reported mesangiolipidosis but also lipid deposition in the glomerular basement membranes that resembled the changes seen in membranous nephropathy on PAS and methenamine silver stains, similar to findings in our patient. Ultrastructurally, there were occasional mesangial lipid deposits and numerous intramembranous, subepithelial, and sub-endothelial lipid deposits. Lipid deposits can also be present in the tubular basement membranes.⁶ Similar findings of diffuse glomerular basement membrane thickening and subepithelial “spikes” on methenamine silver stain were also reported in 2 of 4 cases by Russo *et al.* and in 1 case by Davis *et al.*^{8,10}

The most striking pathologic finding in advanced ALGS is the presence of numerous lipid vacuoles (lipidosis) embedded within the glomerular basement membrane (GBM), which is identical in appearance to familial lecithin–cholesterol acyltransferase (LCAT) deficiency. Lecithin–cholesterol acyltransferase is required for the esterification of cholesterol, and deficiency in this disorder leads to excess free cholesterol and lecithin in the blood. In the kidney, this has been

Table 2. Teaching points regarding Alagille syndrome

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| Chronic kidney disease is common in patients with Alagille syndrome, and 40% of patients have renal abnormalities. |
| There is a spectrum of histologic renal findings in Alagille syndrome, including renal dysplasia that likely results from mutations in the Notch signaling pathway. |
| Other renal histologic findings include mesangiolipidosis and bile cast nephropathy, highlighting the harmful effects of defects in the liver on the kidney |

reported to result in subepithelial lipid deposition in the glomerular basement membranes resembling membranous nephropathy, deposition in podocyte cytoplasm, deposition in tubular basement membranes, and deposition in capillary endothelial cytoplasm.^{19,20} In our patient, the lipid vacuoles could be observed in direct contact with either the capillary lumen or the podocyte cell membrane, which demonstrates these sizeable objects traversing the GBM. Although red blood cells must also traverse the entire thickness of the GBM, this has rarely been reported in thin basement membrane nephropathy.²¹ Although hyperlipidemia is characteristic of nephrotic syndrome, GBM lipidosis has never been seen in the setting of minimal change disease, focal segmental glomerulosclerosis, or membranous nephropathy. Therefore, additional aberrations involving lipid biology may be necessary.

In conclusion, our case illustrates many key histopathologic features of Alagille syndrome: renal dysplasia due to the disruption of the Notch signaling pathway in early nephron development and glomerular, tubular basement membrane, and vascular lipid deposition as a consequence of cholestasis resulting from bile duct dysplasia in the liver (Table 2). In addition, our case also demonstrates bile cast nephropathy, further highlighting the harmful cumulative impact of liver dysfunction in the kidney.

DISCLOSURE

All the authors declared no competing interests.

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