Infantile nephropathic cystinosis

MB Stokes[1], S Jernigan[2] and VD D'Agati[1]

[1]Department of Pathology, Columbia University College of Physicians and Surgeons, New York, New York, USA and [2]Children's Healthcare of Atlanta at Scottish Rite, Atlanta, Georgia, USA

CASE PRESENTATION

A Caucasian girl, the product of a non-consanguineous union, was delivered after a full-term uneventful pregnancy with a birth weight of 4.1 kg. At 15 months, she presented to the emergency room with signs of a respiratory illness and was found to weigh 6.14 kg, below the 3rd percentile for age. She had no history of fevers, dehydration, or photophobia and there was no family history of renal or other medical disease. The patient had one elder sister who had no medical complaints. Physical examination revealed a fair-haired blue-eyed child with pale complexion. Details of her ethnic ancestry were unknown. Blood pressure was normal and the remainder of the physical examination was also unremarkable.

Laboratory investigations at 15 months revealed blood urea nitrogen 9 mg per 100 ml (normal range (N) = 8–25), serum creatinine 0.6 mg per 100 ml (estimated creatinine clearance <60 ml min^{-1}), serum sodium 133 mmol l^{-1} (N = 136–145), potassium 3 mmol l^{-1} (N = 3.5–5.5), chloride 105 mmol l^{-1} (N = 96–106 mEq l^{-1}), total carbon dioxide (CO_2) 18.7 mEq l^{-1} (N = 22–30), serum calcium 10.6 mg per 100 ml (N = 8.5–10.6 mg per 100 ml), and phosphate 3.4 mg per 100 ml (N = 2.5–4.6). Arterial blood gas was not drawn. Urinalysis revealed specific gravity <1.005, no protein or cells, and trace glycosuria. However, there was no evidence of phosphaturia (24 h urinary phosphorous excretion 0.2 mmol, N = 0.4–1.3), hypercalciuria, or increased urinary organic acid excretion. Measurement of urinary amino acids was not performed. Hematologic parameters and thyroid function tests, including thyroid stimulating hormone, T4, and free T4, were within normal limits. Based on the finding of hypokalemia, an investigation for possible Bartter's syndrome was undertaken. These tests revealed markedly elevated plasma levels of renin (119 853 ng per 100 ml per h, N = 171–115) and aldosterone (715 ng per 100 ml, N = 3–35) that, together with the findings of normal blood pressure, hypokalemia, and hyponatremia, suggested a diagnosis of acquired Bartter's syndrome.

The patient was treated for the next two and a half years with indomethacin and electrolyte replacement, including potassium chloride and calcium carbonate. During this time, she gained weight and normalization of blood electrolytes. However, at 4 years of age, she was noted to have worsening renal insufficiency (serum creatinine 1.2–1.4 mg per 100 ml) and 4+ proteinuria on dipstick. At this time, an elevated thyroid stimulating hormone was noted (17.27 IU ml^{-1}, N = 0.3–5.0), indicating hypothyroidism, and thyroid replacement therapy was commenced. Indomethacin was discontinued but renal function and proteinuria continued to deteriorate over the following year. At age 5 years, serum creatinine was 2.9 mg per 100 ml, urinary protein excretion was 1.8 g per 24 h, serum albumin was 3.4 g per 100 ml (normal >2.5 g per 100 ml), and blood pressure was 80/50 mm Hg. A renal biopsy was performed.

RENAL BIOPSY

By light microscopy, there were 97 glomeruli, 78 of which showed complete or near-complete global sclerosis and 7 of which showed segmental obliteration of capillary lumens by sclerosis and hyalinosis. Most of the non-sclerotic glomeruli displayed occasional multinucleated visceral and parietal epithelial cells, without associated endocapillary hypercellularity or crescent formation (Figures 1–3). The multinucleated visceral epithelial cells expressed vimentin and CD10, but were CD68 negative, consistent with a podocyte origin. No mitotic figures or immunoperoxidase staining for Ki-67 (a marker of cell proliferation) was seen in these cells. There was focal prominence of the juxtaglomerular apparatus. Moderate to severe tubular atrophy and interstitial fibrosis affected 65% of the cortical area. Numerous interstitial CD68+ histiocytes were seen (Figure 4). Arterial vessels showed mild to moderate medial hypertrophy. Immunofluorescence microscopy showed segmental glomerular tuft staining in 5 of 15 glomeruli for IgM, kappa and lambda, and no specific staining for IgG, IgA, complement (C3 or C1q), fibrinogen, or albumin. The tissue for immunofluorescence studies had been immersed in Zeus fixative; thus, no unfixed cryostat tissue sections were

Correspondence: *MB Stokes, Department of Pathology, Renal Pathology Laboratory, VC14-224, Columbia University College of Physicians and Surgeons, New York, New York 10032, USA. E-mail: mbs2101@columbia.edu*

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available for examination under polarized light microscopy. However, toluidine blue-stained screening sections for electron microscopy revealed numerous clear intracytoplasmic clefts within interstitial cells, corresponding to the CD68+ histiocytes seen in formalin-fixed tissue sections (Figure 5). Ultrastructural examination demonstrated empty needle- and rhomboid-shaped inclusions within these cells, some of which were limited by a single membrane (Figure 6). No cytoplasmic inclusions were seen in interstitial endothelial cells, tubular epithelium, or arterial wall myocytes. However, the electron microscopy tissue sample contained sclerotic glomeruli only, precluding evaluation of glomerular cells.

DIAGNOSIS

The pathologic findings of chronic tubulo-interstitial nephropathy with numerous multinucleated podocytes and cytoplasmic crystalline inclusions within interstitial cells favored a diagnosis of nephropathic cystinosis.

CLINICAL FOLLOW-UP

Following renal biopsy examination, testing performed in peripheral blood leukocytes revealed markedly elevated cystine levels (6.1 mmol ½ cystine per mg protein; control <0.1 mmol ½ cystine per mg protein), confirming the diagnosis of cystinosis. Ophthalmologic examination to detect corneal crystals was not performed as the patient had no visual symptoms. Treatment with cysteamine was started immediately and the patient was listed for renal transplantation.

DISCUSSION

Cystinosis is a rare autosomal recessive disease caused by heritable mutations of the *CTNS* gene encoding cystinosin, a lysosomal transport protein. Defective lysosomal transport leads to widespread accumulation and crystallization of cystine in many organs, notably kidney, cornea, bone marrow, thyroid, lymph nodes, liver, and spleen.[1] Renal manifestations dominate the clinical presentation and course in infantile nephropathic cystinosis, the most common and severe phenotype (see Table 1). Other organs frequently affected include the cornea and thyroid, causing painful photophobia and hypothyroidism, respectively. A milder cystinosis phenotype presents in adolescence with more slowly progressive renal failure and ocular disease, and an adult ocular (non-nephropathic) form manifests only corneal disease, and never develops signs of renal or other organ involvement.[1] Infantile nephropathic cystinosis is associated with two severe *CTNS* mutations, including deletions, insertions, nonsense, missense, and splicing mutations,[1] that cause complete abolition of cystine transport. The adolescent and ocular forms generally have one severe and one mild mutation, leading to reduced transport. The sparing of the kidney in cases of ocular cystinosis may reflect tissue-specific expression of splicing factors, or the increased endogenous level of *CTNS* mRNA normally seen in the kidney.[2] Individuals who are heterozygous for severe *CTNS* mutations demonstrate elevated levels of leukocyte cystine but are completely asymptomatic.

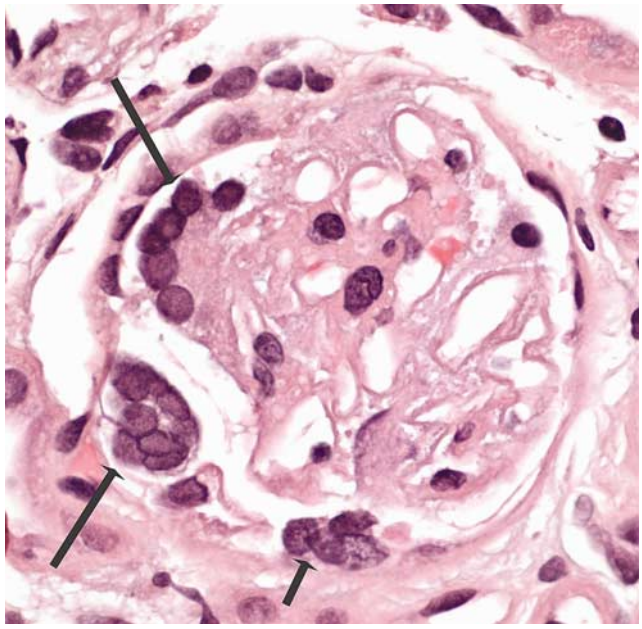

Figure 1 | Glomerulus with multiple multinucleated podocytes and parietal epithelial cells (arrows) (hematoxylin and eosin stain; original magnification ×40).

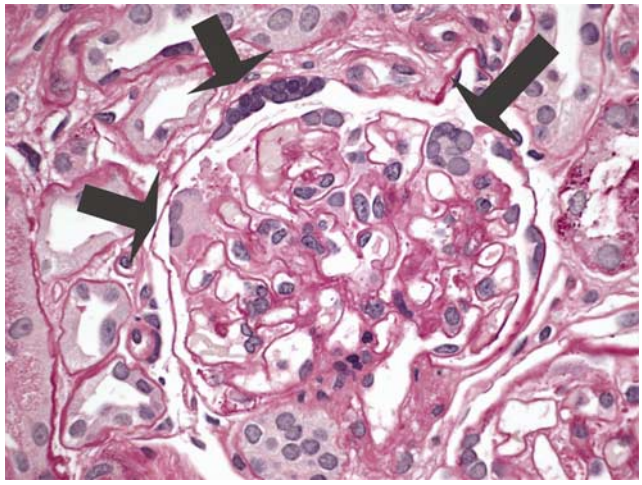

Figure 2 | Periodic acid–Schiff (PAS) reaction demarcates the glomerular basement membrane and highlights the visceral and parietal epithelial location of the multinucleated cells (arrows) (PAS; original magnification ×40).

Most patients with infantile nephropathic cystinosis present during the first year of life with failure to thrive, polyuria, polydipsia, and/or dehydration and are found to have Fanconi's syndrome with normal anion gap metabolic acidosis.[1] Some of these patients may develop vitamin D-resistant rickets due to phosphaturia. Renal function is generally normal at presentation, but without treatment most individuals progress to end-stage renal disease after 5 years of

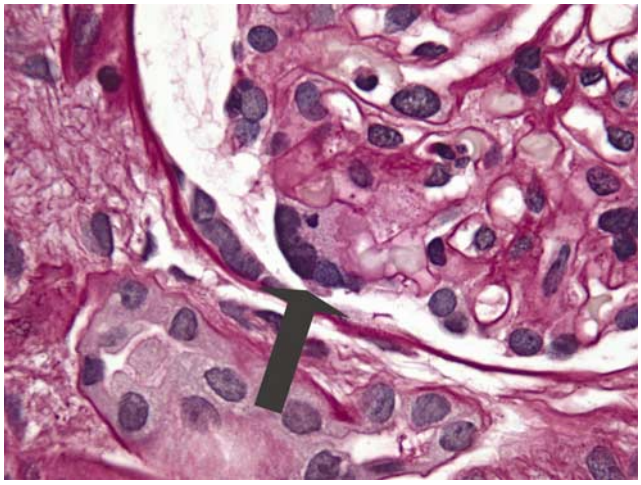

Figure 3 | High-power image of multinucleated visceral epithelial cell (arrow) (PAS reaction; original magnification ×60).

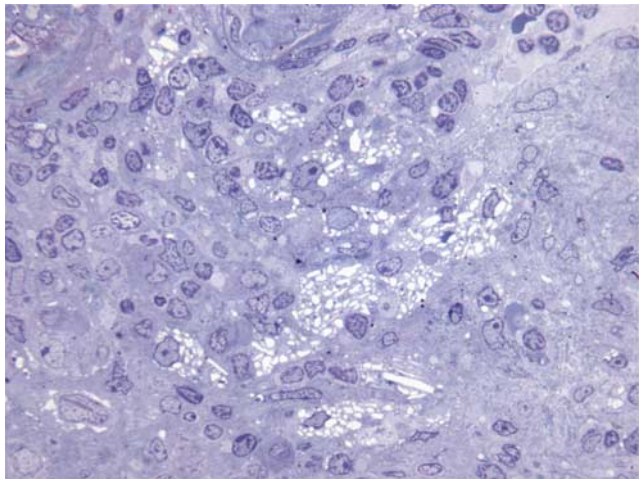

Figure 5 | Interstitial cells display cytoplasmic clear clefts consistent with crystalline inclusions (toluidine blue stain; original magnification ×100).

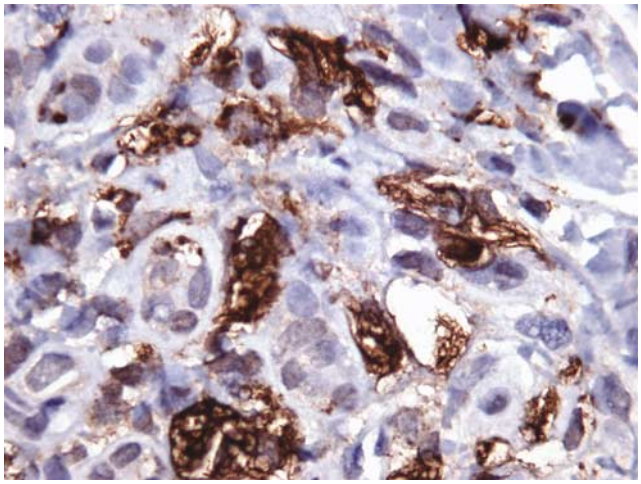

Figure 4 | Interstitial histiocytes (brown stain) displaying cytoplasmic clefts and inclusions (CD68 immunoperoxidase stain; original magnification ×60).

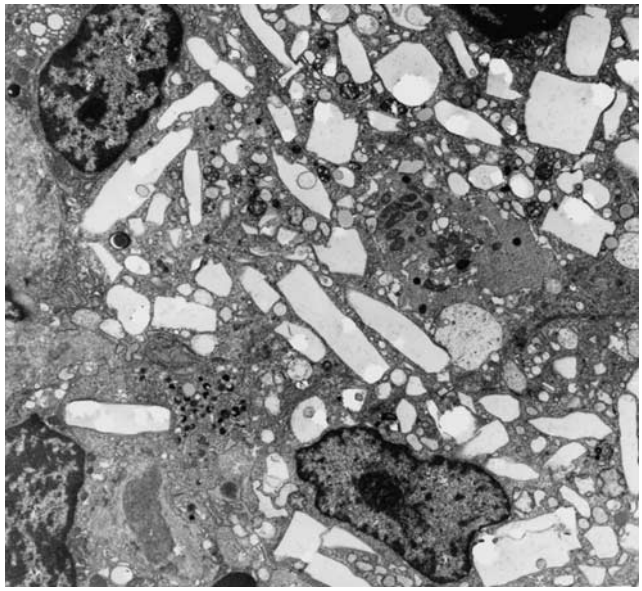

Figure 6 | Interstitial cells containing multiple clear, elongated, needle-shaped and rhomboid cytoplasmic inclusions (electron photomicrograph; original magnification ×3000).

age, and only rarely before then.[3] The urine and serum biochemical abnormalities in Fanconi's syndrome, including hyperchloremic metabolic acidosis, hypokalemia, hypophosphatemia, glycosuria, aminoaciduria, and phosphaturia, are consistent with a generalized defect in proximal tubular transport.[4] There have also been several reported cases of nephropathic cystinosis presenting with features of secondary Bartter's syndrome (hypokalemia, hypochloremic metabolic alkalosis, hyperreninemia, and hyperaldosteronism), suggesting abnormalities of sodium and chloride reabsorption.[5–11] Most of these cystinosis patients also had Fanconi's syndrome and, similar to the present case, several showed reversal of Bartter's syndrome following treatment with aspirin or indomethacin, consistent with an acquired defect.[7,8,10] Our patient presented with an unusual constellation of abnormalities including glycosuria and metabolic acidosis, consistent with partial Fanconi's syndrome, as well as biochemical features suggestive of acquired Bartter's syndrome, including hypokalemia, hyponatremia, normotensive hyperreninema, and hyperaldosteronemia.

The pathophysiology of tubular transport defects in cystinosis patients is poorly understood, reflecting the lack of an animal model for this disease. Knockout mice lacking cystinosin (*CTNS*−/−) do not develop signs of Fanconi's syndrome, despite accumulation of lysosomal cystine in proximal tubules.[12] Investigations using cystine-loaded proximal tubular epithelial cells have demonstrated depletion of free phosphate and defective ATP production and inhibition of Na^+-dependent transporters.[4,13,14] It has been

Table 1 | Clinical manifestations of infantile nephropathic cystinosis (adapted from Gahl *et al.*[1])

At presentation
Common
Failure to thrive
Polydipsia and polyuria
Fanconi's syndrome
Vitamin D-resistant rickets
Progressive renal failure
Photophobia
Hypothyroidism
Uncommon[a]
Bartter's syndrome
Nephrotic syndrome
Diabetes insipidus
Post-renal transplantation
Dysphagia
Myopathy
Exocrine pancreatic insufficiency
Diabetes mellitus
CNS deterioration
Primary hypogonadism

[a]May be transient and coexist with common manifestations.

suggested that abnormal proximal tubular Na^+/K^+ ATPase function due to ATP depletion might lead to increased distal tubular sodium delivery and Bartter's syndrome in some cystinosis patients.[5]

The diagnosis of cystinosis is confirmed by demonstrating markedly elevated cystine levels in peripheral blood leukocytes.[1] Detection of corneal crystals by slit-lamp examination is virtually diagnostic of cystinosis in the setting of childhood Fanconi's syndrome because these crystals are not seen with other genetic causes, such as tyrosinemia, galactosemia, hereditary fructose intolerance, and hepatorenal glycogenosis (Fanconi–Bickel syndrome), or with acquired causes of Fanconi's syndrome, such as lead poisoning. However, corneal crystals may not develop for several years, and therefore this finding is not sensitive for early diagnosis. Genetic testing reveals that the majority of European patients with cystinosis have a homozygous 57-kb deletion of the first 10 exons of the *CTNS* gene, consistent with a founder effect.[15,16] However, over 50 different mutations have been linked to cystinosis and therefore genetic testing for cystinosis is generally not practicable, except in families known to have the 57-kb deletion.[1] The association of cystinosis with fair hair and blue eyes described in many reported cases likely reflects the occurrence of founder mutations in the Northern European population several hundred years ago,[1] rather than any direct pathogenic relationship to pigmentation, as cystinosis has also been reported in individuals of African descent who do not show these physical characteristics.

The renal pathologic findings in infantile cystinosis consist of a chronic tubulo-interstitial nephropathy, with characteristic findings of multinucleated podocytes and intracellular crystalline inclusions in interstitial histiocytes.[9] There may be an increased number of immature glomeruli.[17] Microdissection studies of isolated nephrons have demonstrated a characteristic 'swan-neck' deformity (due to selective tubular atrophy involving the first part of the proximal tubule) that appears after 6 months but may not be present earlier, suggesting that this lesion is acquired rather than congenital.[17] The 'swan-neck lesion' may be preceded by the presence of small intracellular crystals, consistent with cystine, within tubular epithelial cells.[17] However, the 'swan-neck lesion' reportedly also occurs with other causes of Fanconi's syndrome and is not detectable by routine light microscopic examination, limiting its diagnostic utility. Because cystine crystals are water-soluble, these are not retained in tissue sections following routine histologic preparation with aqueous solutions.[18] The cystine crystals are birefringent under polarized light in alcohol-fixed tissue or in unfixed frozen tissue. In our case, crystals were not detectable under polarized light microscopy in cryosections because the frozen tissue had been placed in Zeus fixative prior to freezing. In toluidine blue-stained sections of epoxy resin-embedded tissue, the presence of crystals can be inferred by the appearance of needle-shaped or rhomboid intracytoplasmic clefts. These inclusions are most numerous in interstitial CD68 + histiocytes,[9] as shown in the present case, but have also been described in glomerular epithelial cells and tubular epithelial cells.[17–19] In the present case, the absence of crystalline inclusions in epithelial cells may reflect the advanced chronic injury, as no nonsclerotic glomeruli were available for ultrastructural examination. Spear *et al.*[18] described the presence of distinctive 'dark cells' in plastic-embedded sections prepared for electron microscopy, probably due to a reaction of osmium tetroxide with intracellular cystine, but these were not seen in the present case.[18]

The finding of numerous multinucleated podocytes may be the most characteristic pathologic finding in nephropathic cystinosis, as these cells only rarely occur in other conditions.[9,18,20] However, these cells are not seen in sclerotic glomeruli, hence their absence in cases of severe chronic renal disease does not exclude a diagnosis of cystinosis. We also identified multinucleated cells lining Bowman's capsule, but whether these cells correspond to the recently described 'parietal podocytes' is unclear.[21] Podocytes have limited potential for cell division (with the notable exception of collapsing variant focal segmental glomerulosclerosis, and some cases of crescentic glomerulonephritis) and multinucleated podocytes were detected only in rare glomeruli in 6 of 164 renal biopsies of diverse renal disease where these were specifically sought.[20] The lack of CD68 staining in the present case, and in one other case report,[9] provides evidence against a histiocytic origin. The pathogenesis of multinucleated podocytes probably involves aberrant nuclear division without accompanying cytokinesis in injured podocytes, a finding previously described in some patients with focal segmental glomerulosclerosis.[20] It was the observation of distinctive multinucleated podocytes, together with the findings of intracellular clefts in interstitial cells, that led to a pathologic diagnosis of nephropathic cystinosis in the present case.

The differential diagnosis of intracellular inclusions in renal biopsy specimens includes hereditary disorders of peroxisomal function, such as Refsum disease, Zellweger's syndrome, and primary hyperoxaluria, glycogen storage disease (for example, galactosemia), and hereditary lipid storage disorders, such as Fabry disease, mucolipidopsis, and Gaucher disease. In older individuals, the differential diagnosis is broader and includes secondary hyperoxaluria (such as due to ethylene glycol ingestion, enteric disease, vitamin C intoxication, or excess ingestion of oxalate-containing foods) and dysproteinemia, which may manifest as crystalline monoclonal light chain inclusions in tubular epithelial cells, glomerular epithelial cells, or interstitial histiocytes. Unlike cystine crystals, oxalate crystals display birefringence under polarized light in formalin-fixed paraffin-embedded tissue sections. Monoclonal light chain crystalline inclusions are detectable by routine immunofluorescence microscopy in frozen tissue sections, but pronase digestion of formalin-fixed tissue sections may be a more sensitive technique (due to antigen retrieval).

The management and treatment of infantile nephropathic cystinosis involves supportive therapy to maintain fluid balance and replace electrolyte losses in the initial stage. Early recognition of the diagnosis and specific treatment with oral cysteamine (a cystine-depleting agent) may delay the onset of renal failure and other organ involvement, including thyroid disease.[1] Cysteamine eye-drops may alleviate visual symptoms by dissolving the corneal cystine crystals. Renal transplantation is associated with increased survival in cystinosis patients who develop end-stage renal disease. Of note, cystine accumulation has been reported in the transplanted kidney but, to date, renal clinical manifestations of cystinosis have not.[22] However, extrarenal clinical manifestations of cystinosis may continue to occur (Table 1).

With worsening renal failure, signs of Fanconi's syndrome may diminish in cystinosis patients, and heavy proteinuria due to hyperfiltration in remnant glomeruli may occur.[9] While we cannot fully exclude the possibility that our patient may have had transient full Fanconi's syndrome prior to her initial presentation, this case emphasizes that the presentation of nephropathic cystinosis may not fulfill all criteria for either Fanconi's syndrome or Bartter's syndrome, and may present with incomplete and mixed features of both syndromes. A high index of clinical suspicion is needed for diagnosis of nephropathic cystinosis in these children, as early initiation of therapy with cysteamine may slow the rate of disease progression. Awareness of the characteristic pathologic findings of multinucleated podocytes and crystal-containing interstitial cells may suggest the correct diagnosis in subjects who present with atypical clinical features.

DISCLOSURE

The authors have no conflicts of interest to disclose.

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