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Brief Communication

The incidence of atubular glomeruli in nephropathic cystinosis renal biopsies

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ABSTRACT

Nephropathic cystinosis results from lysosomal cystine storage and, if untreated with cysteamine, results in end-stage renal disease by 10 years of age. The renal Fanconi syndrome occurs in the first year of life and is accompanied by a characteristic "swan neck" deformity of the proximal renal tubule. The linkage between cystine storage, Fanconi syndrome, and renal failure has not been understood. This study reports the presence of substantial numbers of atubular glomeruli (ATG) in end-stage cystinotic renal tissue. Compared to normal renal tissue, cystinotic kidneys at end stage had 69% atubular glomeruli and 30% atrophic glomeruli. Normal renal tissue had 4% ATG and 0% atrophic glomeruli ($p<0.0001$ for both comparisons). These nonfunctioning nephrons may be the end result of cell loss from the tubules and represent the final stage of the swan neck deformity. The process is consistent with the previously reported increased apoptosis in renal tubule cells due to lysosomal cystine storage.

1. Introduction

Nephropathic cystinosis is a lethal inborn error of metabolism characterized by intralysosomal storage of the disulfide amino acid cystine. The ramifications of lysosomal cystine storage are still being explored; however, the phenotype, characterized by short stature, retinopathy, failure to thrive, the renal Fanconi syndrome, hypothyroidism, and ultimately renal death by the first decade of life, is well known [1]. The pathogenesis of both the renal Fanconi syndrome and ultimately renal failure in this condition is unknown. How lysosomal cystine produces cell injury and death is not established. Normally, cystine exits lysosomes into the cytosol by means of cystinosin, the lysosomal cystine transporter, which is mutated in cystinosis [2]. When the activity of this protein is ablated by mutation, cystine has no means to exit the lysosome except for exocytosis, depositing cystine in the extracellular fluid. Thus, cystine inside cystinotic lysosomes has no means of communication with the cytosol, and hence, no means to damage cellular metabolism. In recent times, several authors have expanded on an idea first proposed by Patrick in 1965 [3], that cystine could alter the intracellular redox balance, or "poison" critical thiol moieties of varies enzymes, thus inhibiting them and damaging metabolic pathways [4–7]. Since it is now known that cystine is isolated inside lysosomes, these hypotheses must also explain how cystine can interact with cytosolic components to lead to the observed pathologic changes. However, a recent publication has examined the redox couple in cystinotic fibroblasts and found no evidence of perturbed thiol/disulfide equipoise [8].

An alternate explanation has recently been suggested. It has been shown in cultured cystinotic fibroblasts and proximal renal tubule cells that the presence of lysosomal cystine augments the rate of apoptosis 200% to 300% [9–12]. This push toward inappropriate cell death could explain the relatively long period required for end-stage renal disease to develop. The usual patient with untreated cystinosis has normal renal function at birth with renal failure developing in most by age 10 [1]. The mechanism of increased apoptosis appears to be due to an increase in the activity of protein kinase C-δ (PKC-δ) [11], a known proapoptotic modulator, which, upon cysteinylation, occurring from release of lysosomal cystine early in the apoptotic cascade, greatly increases its activity. This increased predisposition to cell death due to lysosomal cystine storage would, over time, result in a generalized hypocellularity leading to short stature and other phenotypic findings. It is noteworthy that both retina and proximal renal tubule cells are among the first affected tissues in cystinosis [1] and also among those known to be highly sensitive to apoptosis [13,14]. This would then explain the appearance of renal Fanconi syndrome as the result of loss of renal proximal tubular epithelial cells such that adequate reabsorption of water and small molecules is impaired.

In addition, the continued loss of these proximal tubular epithelial cells could explain a morphologic hallmark of cystinosis, the so-called swan neck deformity, first described as pathognomonic of cystinosis in the middle of the last century [1,15]. This change is described as shortening and atrophy of the earliest portion of the proximal convoluted tubule. Progression of proximal tubule cell loss, caused by inappropriate apoptosis, could account for this altered appearance.

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Recent studies have defined the presence of atubular glomeruli in chronic renal disease in which the glomerulus becomes detached from the proximal tubule, leaving a nonfunctional nephron. A wide range of diseases have been shown to result in an increased proportion of these atubular glomeruli including: glomerular diseases (e.g., IgA nephropathy, congenital nephrotic syndrome, diabetes mellitus), tubular disorders (e.g., transplant rejection, pyelonephritis, obstructive nephropathy), toxin-mediated nephropathy, and ischemic disease in renal artery stenosis [16]. The fact that such a wide spectrum of diseases leads to this irreversible change underscores the vulnerability of the proximal tubular cell to injury.

We hypothesized that patients with cystinosis would develop atubular glomeruli as increasing numbers of proximal tubules lost epithelial cells causing the tubule to become disconnected from the glomerulus. To test the hypothesis, we collected renal specimens from children with nephropathic cystinosis and evaluated the percentage of atubular and atrophic glomeruli compared to age-matched controls from normal individuals.

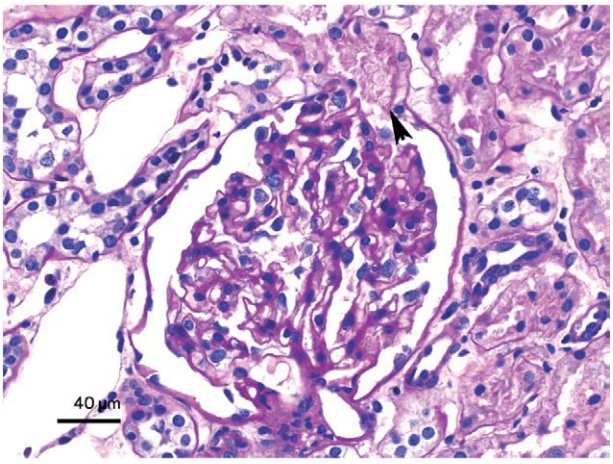

Fig. 1. Glomerulus with a normal tubule exit site (arrowhead).

2. Methods

2.1. Patient recruitment

This study was approved by the University of Michigan's Institutional Review Board. To preserve anonymity of the patients, they were approached indirectly using the Cystinosis Research Network, a patient organization for children and adults with cystinosis, to publicize the availability of the cystinosis tissue repository at the University of Michigan. The patient's physician , or the pathologist who had custody of the sample, would contact the senior author (JGT) informing him that a renal biopsy or transplant specimen was available from a patient with cystinosis. The sample was then deidentified and given a local code to permit retrieval of the sample if a future patient need arose and sent to the repository. After accession, the tissue was forwarded for analysis to the renal pathologists (CL and PW) as described below. Because of the need for indirect recruitment of samples, extensive case histories are not available.

2.2. Control samples

Renal cortices from two noncystinotic control patients with isolated proteinuria were studied for comparison. The control biopsies showed no significant histopathologic abnormalities by light microscopy. Immunofluorescence was negative for IgG, IgA, IgM, C3, C4, C1q, fibrinogen, kappa, and lambda light chains. Electron microscopy revealed unremarkable capillary loops with moderate foot process effacement. No electron-dense deposits were present, and there was no hypercellularity.

2.3. Tissue processing

The tissue was received fixed in formalin and embedded in paraffin at the local site using standard pathology protocols. The blocks were cut at 4 µm, producing 40 slides, each containing two sections, for a total of 80 sections per sample. The tissue was then reacted with periodic acid–Schiff (PAS) reagent, and the glomeruli were studied on serial sections to evaluate the glomerulotubular junction (GTJ).

2.4. Analysis

Only glomeruli which were complete in their entirety were included in the study. Globally sclerotic glomeruli and those with incomplete Bowman's capsule were excluded. Bowman's capsule was examined in each section to identify the GTJ. GTJ were classified as either normal (Fig. 1), atrophic (Fig. 2), or atubular (Fig. 3). Atrophic tubules were defined as those with flattened epithelium and no brush border at the GTJ. Atubular glomeruli were defined as those in which there was no tubular connection with Bowman's capsule. A photomicrograph of each glomerulus was taken at each level of section to confirm and document the type of GTJ present.

2.5. Apoptosis detection

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was performed on 4-µm-thick tissue sections from two of the cystinosis cases and two control patients with minimal histological changes by light, immunofluorescence, and electron microscopy. The TUNEL system from Promega Corporation, USA, was used according to the manufacturer's instructions and examined by light microscopy. For each case, the number of positively staining tubular nuclei was counted in 25 fields at 200× and expressed as number of positive cells per 25 high-power fields (HPFs).

2.6. Statistical methods

Comparison between groups was performed using Fisher's exact test. This tests statistical significance between categorical variables and is used in place of the χ^2 test when sample variables are small. A p value <0.05 was considered to be significant.

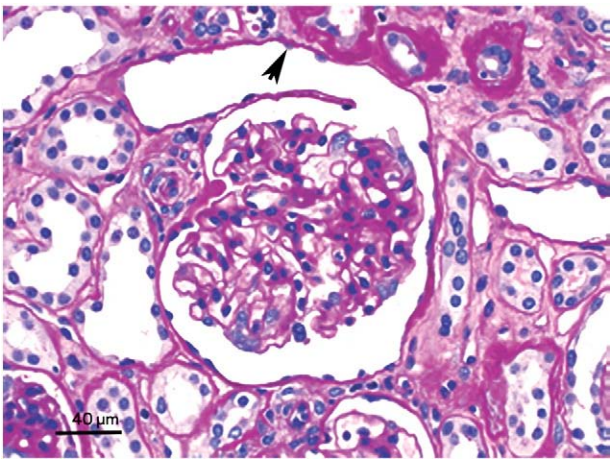

Fig. 2. Glomerulus with an atrophic tubule from cystinotic patient. The tubular epithelial cells are flattened and lack the brush border (arrowhead).

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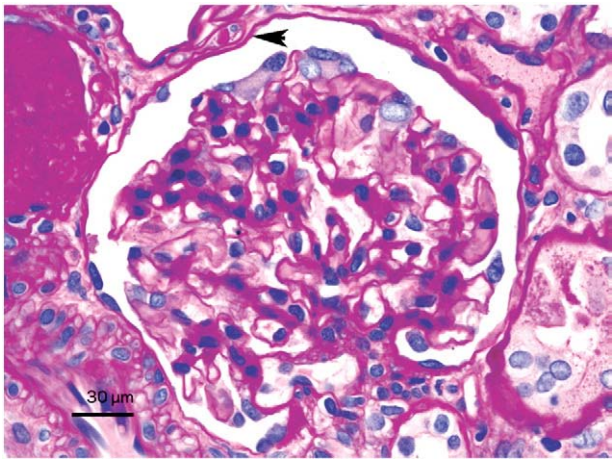

Fig. 3. Atubular glomerulus from a cystinotic patient. The glomerular tuft is intact; however, no tubular connection with Bowman's capsule was identified on serial sections. Note the slight thickening of Bowman's capsule at the side opposite the vascular pole where the tubule should be (arrowhead).

2.7. Patients

2.7.1. Case 1

This boy had a renal transplant at age 10 years having been diagnosed with nephropathic cystinosis at age 5 years. From the time of diagnosis until real transplantation, he had been maintained on optimal doses of cysteamine and displayed excellent compliance. At transplantation, the native kidneys were removed. Representative kidney samples obtained as a part of the routine pathological analysis were fixed in formalin, and processed into paraffin. One typical block was sent for serial section analysis.

Cystine content was also determined in the renal tissue. There was 530 nmol/mg protein in the renal cortex and 740 nmol/mg protein in the medulla, consistent with previous such measurements in cystinotic renal tissue [1].

2.7.2. Case 2

No clinical information is available on this sample from kidneys removed at renal transplantation for ESRD due to cystinosis at 21 years of age. The pathology report included extensive glomerulosclerosis, interstitial lymphocytic inflammation, and calcifications with tubular atrophy.

2.7.3. Case 3

This patient came to renal transplantation due to end-stage renal disease at age 24 years. He had been diagnosed at age 13 months and, at the time of transplantation, was receiving Cystagon 2400 mg/day divided q6h. His creatinine had risen to 2.8 mg/dL at 23 years of age, and he underwent renal transplantation at 24 years.

2.7.4. Case 4

At the time of bilateral nephrectomy, this girl was 13 years old, weighed 36 kg, and was 145-cm tall. Her creatinine pretransplant was 2.0, and BUN was 30. She was treated for hypertension and was receiving oral Cystagon and cysteamine eye drops.

2.7.5. Case 5

This 19-year-old male with cystinosis received a living related donor allograft in 2009. At the time of transplant, his creatinine was 3.09 mg/dL and BUN was 50 with normal electrolytes.

2.7.6. Case 6

This male patient was 18 years old when he had a double nephrectomy for ERSD due to cystinosis.

3. Results

3.1. Atubular glomeruli analysis

A total of 175 glomeruli were suitable for evaluation in the six cystinotic kidney biopsies with 14 glomeruli from the smallest biopsy and 50 from the largest. Seventy-one glomeruli were studied from the control cases. The light microscopic findings in the cystinosis cases were typical of the disease including interstitial fibrosis, tubular atrophy, and widespread glomerular solidification. The characteristic glomerular finding of multinucleated podocytes was also frequently seen in the intact glomeruli. See Figs. 1–3 for representative sections of normal, atrophic, and atubular glomeruli.

As seen in Table 1, the cystinosis patients showed 69% atubular glomeruli in the six renal samples available for study, and 30% atrophic glomeruli, as opposed to 4% ATG and no atrophic glomeruli in the two normal controls. This was significant at the 0.0001 level of chi square analysis.

3.2. Apoptosis

Apoptosis was evaluated using the TUNEL assay. No difference was seen between the renal tissue samples from cystinosis patients at end-stage renal disease and the controls. The kidney samples from patients with cystinosis had a mean of 8 TUNEL-positive cells/25 HPF, while the samples from control patients showed a mean of 7.5 positive cells/25 HPF.

4. Discussion

In the six cases studied, the patients had nephropathic cystinosis, and their native kidneys survived to between 10 and 24 years. As noted above, due to the nature of specimen collection, more detailed histories are not available. However, this study permits a comparison of atubular glomeruli in renal specimens taken from children with cystinosis between ages 10 and 24 years.

The progression of renal disease in cystinosis is characterized by the onset of the renal Fanconi syndrome before 12 months of age with massive polyuria, and salt and water and other small molecule wasting [1]. This is followed by a slow decline in glomerular filtration such that for populations of untreated cystinotic patients, the mean age at complete renal failure is approximately 10 years. Mahoney and Striker [15] recently demonstrated that the swan neck deformity occurs in the first 18 months of life. This current work expands their observation by showing that the apparent end stage of the swan neck deformity is atubular glomeruli. This process effectively disconnects the glomeruli from the excretory system, leading to a failure of filtration for that nephron. Widespread atubular glomeruli then result in failure of filtration for the organ as a whole, and outright renal failure consistent with the time course noted for the disease.

Increased apoptosis resulting from lysosomal cystine storage has been demonstrated in cultured human renal proximal tubular epithelial

Table 1
The incidence of ATG and atrophic GTJ in six cystinotic and two normal renal biopsy samples. *P* values between normal and cystinotic tissue for each category were calculated as described.

	Glomeruli with normal GTJ	Glomeruli with atrophic GTJ	Atubular glomeruli
Cystinosis patients	2/175 (1%)	53/175 (30%)	120/175 (69%)
Range	0–4%	0–56%	44–98%
Normal controls	68/71 (96%)	0/71 (0%)	3/71 (4%)
Range	95–97%	0%	3.3–4.9%
p	<0.0001	<0.0001	<0.0001

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cells. The mechanism is still under investigation, however it appears that cysteinylation of the proapoptotic modulator PKC-δ, resulting from release of lysosomal cystine, leads to an increase in its enzymatic activity [11]. Such increased activity could mediate inappropriately increased apoptosis, leading to progressive cell death in the renal proximal tubule resulting in atubular glomeruli and ultimately renal failure. The demonstration of widespread ATG and atrophic glomeruli in cystinotic renal tissue at end stage in this study supports this concept. There was no correlation between age of patient when the sample was obtained and the incidence of ATG ($r = -0.3$) or atrophic glomeruli. Plasma creatinine concentrations were only available for three patients (cases 3, 4, and 5). A plot of their creatinine versus %ATG did not show a significant correlation ($r^2 = 0.42$). This is perhaps not surprising given that the patients had all reached end-stage renal disease when transplantation was performed. The same reasoning applies to not finding increased apoptosis in these specimens, which is attributed to the rapid nature of that event, such that actually observing a cell undergoing apoptosis is unlikely, and apoptosis is presumed to have already occurred in these patient's tissues. One would expect increased apoptosis to be present much earlier in the disease progression.

If the effect of increased apoptosis in participating in generation of the cystinotic phenotype is born out, then these findings may also have therapeutic implications, as study of antiapoptotic drugs in both chronic and acute diseases is now underway [17]. Other therapeutic agents may be developed, which, in combination with cysteamine bitartrate, a cystine-lowering agent, could, by delaying proximal tubule cell loss, and hence delaying the progression to ATG, further prolong survival of the native kidney in cystinosis.

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