Urinary Protein Excretion Pattern and Renal Expression of Megalin and Cubilin in Nephropathic Cystinosis

Martijn J. Wilmer,[1] Erik I. Christensen, MD, PhD,[2] Lambertus P. van den Heuvel, PhD,[1] Leo A. Monnens, MD, PhD,[3] and Elena N. Levtchenko, MD, PhD[1,4]

Background: Nephropathic cystinosis is the most common cause of inherited renal Fanconi syndrome, caused by mutations in lysosomal cystine carrier cystinosin that result in lysosomal cystine accumulation throughout the body. How defects in cystinosin cause proximal tubular dysfunction is not known. We hypothesized that cystine accumulation could cause disturbed proximal tubular endocytosis by megalin and cubilin.

Study Design: Megalin, cubilin, and their ligands were studied in kidney tissue by means of immunohistochemistry. Urinary protein excretion pattern was evaluated.

Setting & Participants: Kidney tissue from a patient with cystinosis was compared with minimal change nephrotic syndrome tissue, end-stage renal disease tissue, and control renal tissue. Urine from 7 patients with cystinosis was compared with 6 control samples.

Results: Expression of megalin, cubilin, and ligands (transferrin, albumin, vitamin D–binding protein, α_1-microglobulin, retinol-binding protein, and β_2-microglobulin) in convoluted proximal tubules of cystinotic kidney was similar to that in other kidney specimens. In straight tubules, low-molecular-weight proteins were present in only cystinotic kidney samples. Next to low-molecular-weight proteins and albumin, urinary excretion of immunoglobulin G was increased in patients with cystinosis with Fanconi syndrome compared with controls. This was already observed at an early age, suggesting enhanced glomerular permeability in patients with cystinosis.

Limitations: This study is essentially observational, and immunohistochemical data are based on 1 cystinotic kidney.

Conclusion: Our findings indicate that low-molecular-weight proteinuria in patients with cystinosis is not caused by decreased megalin and cubilin expression, and glomerular damage might already be present at early stages of the disease.

Am J Kidney Dis 51:893-903.

INDEX WORDS: Cystinosis; Fanconi syndrome; proteinuria; megalin; cubilin; low-molecular-weight proteins.

Editorial, p. 875

Proteinuria is a common feature of patients with diverse nephropathies and generally is divided into glomerular and tubular types according to the size of proteins detected in urine. The glomerular filtration barrier almost completely restricts high-molecular-weight (HMW) proteins (>100 kDa) such as immunoglobulin G (IgG; 150 kDa) and, depending on charge and shape, allows the sieving of only small amounts of intermediate-molecular-weight (IMW) proteins such as albumin (67 kDa). Increased permeability of the filtration barrier allows these proteins to enter the glomerular ultrafiltrate and results in glomerular proteinuria. Glomerular proteinuria may be selective or nonselective. Selective proteinuria is characterized by the predominance of IMW compared with HMW proteins. Tubular proteinuria is characterized mainly by extensive excretion of low-molecular-weight (LMW) proteins (<40 kDa).[1]

In physiological conditions, LMW proteins filtered across the glomerular barrier are almost completely reabsorbed in the renal proximal tu-

From the [1]Laboratory of Pediatrics and Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; [2]Department of Cell Biology, Institute of Anatomy, University of Aarhus, Denmark; [3]Department of Physiology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen, Nijmegen, The Netherlands; and [4]Department of Pediatrics, University Hospital Leuven, Leuven, Belgium.

Received August 30, 2007. Accepted in revised form March 3, 2008. Originally published online as doi: 10.1053/j.ajkd.2008.03.010 on May 2, 2008.

Address correspondence to Martijn J. Wilmer, Laboratory of Pediatrics and Neurology (656), Radboud University Nijmegen Medical Centre, PO 9101, 6500 HB Nijmegen, The Netherlands. E-mail: m.wilmer@cukz.umcn.nl

0272-6386/08/5106-0006$34.00/0
doi:10.1053/j.ajkd.2008.03.010

bule by receptor-mediated endocytosis. Reabsorption of proteins in proximal tubules involves initial binding to the multiligand endocytic receptors megalin and cubilin, highly expressed at the brush-border membrane of proximal tubules collaborating in the uptake of their ligands. Ligands are dissociated from the receptors in intracellular vesicles and degraded further by lysosomal enzymes to LMW fragments and amino acids. Megalin and cubilin are recycled to the apical membrane.[2,3]

Patients presenting with generalized proximal tubular dysfunction, so-called De Toni-Debré-Fanconi syndrome, have LMW proteinuria and albuminuria, together with increased sodium, potassium, and bicarbonate excretion; aminoaciduria; glucosuria; hypercalciuria; hyperuricosuria; and phosphaturia. Nephropathic cystinosis is the most common cause of inherited renal Fanconi syndrome in children, progressing toward renal failure caused by interstitial fibrosis.[4] This autosomal recessive disorder is caused by mutations in the *CTNS* gene (17p13.3) encoding the lysosomal cystine carrier cystinosin, which leads to lysosomal accumulation of cystine in all tissues.[5] Although the genetic defect of cystinosis has been elucidated, the cellular pathways involved in defective proximal tubular reabsorption and, subsequently, end-stage renal disease (ESRD) are enigmatic.

The degree of proximal tubular dysfunction in patients with nephropathic cystinosis is variable. Patients with the most severe infantile cystinosis (Online Mendelian Inheritance in Man [OMIM] identifier 219800) develop full-blown Fanconi syndrome during the first year of life and mainly have truncating mutations in the *CTNS* gene, whereas patients with the intermediate or juvenile form of the disease (OMIM 219900), characterized by less severe proximal tubular dysfunction and slower disease progression rate, generally have milder mutations, allowing some cystinosin activity.[6] In a recent study, 8 of 14 patients with noninfantile cystinosis had Fanconi syndrome, whereas proteinuria was present in all patients.[7]

Defective endocytosis of proteins in proximal tubules was shown to cause LMW proteinuria and albuminuria in several clinical and experimental conditions[8-11] and was documented most extensively in patients with Dent disease.[12,13] This disorder (OMIM 300009) is caused by mutations in the *CLCN5* gene encoding the endosomal chloride-proton exchanger CLC5.[14] Similar to cystinosis, Dent disease manifests with Fanconi syndrome and is characterized further by nephrolithiasis and nephrocalcinosis.[15] Patients with Dent disease and $Clcn5^{-/-}$ mice have decreased megalin and cubilin expression at the brush border of renal proximal tubules, which is suggested to be the cause of LMW proteinuria and albuminuria.[13,16] Furthermore, urinary megalin deficiency in patients with Dent disease and $Clcn5^{-/-}$ mice suggest a defect in the recycling pathway of megalin.[17]

Remarkably, decreased urinary excretion of megalin also was shown in patients with Lowe syndrome.[17] This syndrome (OMIM 309000) is caused by mutations in the OCRL1 (*OCRL*) gene encoding phosphatidylinositol 4,5-biphosphate-5-phosphatase. Patients with Lowe syndrome also develop renal Fanconi syndrome combined with severe psychomotor delay and congenital cataract.[18]

Because proteinuria is a predictor of progression in patients with various renal diseases,[19] we were interested in unraveling the mechanism of proteinuria in patients with cystinosis with progressive tubulointerstitial damage leading to renal failure.[20] We hypothesized that alterations in megalin and cubilin expression could, analogous to Dent disease and Lowe syndrome, cause proteinuria in patients with cystinosis. To investigate this hypothesis, we examined renal tissue and urine from patients with cystinosis for the presence of megalin, cubilin, and their ligands by means of immunohistochemistry and immunoblotting.

METHODS

Patients and Controls

Cystinotic renal tissue was obtained 2 months after renal transplantation from nephrectomized kidney of an 8-year-old boy with infantile nephropathic cystinosis caused by homozygous insertion of a G after nucleotide 922 in the *CTNS* gene (NM_001031681.2:c.922_923insG). Nephrectomy of his native kidney was performed because of persisting Fanconi syndrome with excessive loss of fluid and electrolytes. Before nephrectomy, urinary protein excretion was 83 mg/dL. The renal graft of this patient with cystinosis was lost because of chronic rejection and nephrectomized 5 years after transplantation with signs of ESRD. We used this ESRD graft for immunohistochemistry in this study.

Control renal tissue was obtained from a healthy man aged 43 years. Additionally, renal tissue from an 8-year-old