Urinary Megalin Deficiency Implicates Abnormal Tubular Endocytic Function in Fanconi Syndrome

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Abstract. Normal reabsorption of glomerular filtrate proteins probably requires recycling of the endocytic receptors megalin (gp330) and cubilin. Both receptors are located on the luminal surface of the renal proximal tubule epithelium. Whether abnormal amounts of receptor are present in the urine of patients with Dent’s disease, Lowe’s syndrome, or autosomal dominant idiopathic Fanconi syndrome was explored. They are all forms of the renal Fanconi syndrome and are associated with tubular proteinuria. Urine samples of equal creatinine contents were dialyzed, lyophilized, and subjected to electrophoresis on nonreducing sodium dodecyl sulfate-5% polyacrylamide gels. Proteins were blotted and probed with anti-megalin IgG, anti-cubilin IgG, or receptor-associated protein. Megalin and cubilin levels detected by immunochemiluminescence were measured as integrated pixels and expressed as percentages of the normal mean values. A striking deficiency of urinary megalin, compared with normal individuals (n = 42), was observed for eight of nine families with Dent’s disease (n = 10) and for the two families with Lowe’s syndrome (n = 3). The family with autosomal dominant idiopathic Fanconi syndrome (n = 2) exhibited megalin levels within the normal range. The measured levels of cubilin were normal for all patients. These results are consistent with defective recycling of megalin to the apical cell surface of the proximal tubules and thus decreased loss into urine in Dent’s disease and Lowe’s syndrome. This defect would interfere with the normal endocytic function of megalin, result in losses of potential ligands into the urine, and produce tubular proteinuria.

The endocytic receptors megalin (gp330) and cubilin are expressed in high concentrations in renal proximal tubules, where they mediate the uptake of a wide variety of protein ligands from the glomerular filtrate (1–4). Megalin, which was originally identified in proximal tubule cells by Kerjaschki and Farquhar (5), is a type 1 integral membrane protein with 36 putative extracellular “ligand-binding repeats” and only a small cytoplasmic domain (1,5). In contrast, cubilin lacks a classic transmembrane domain but is thought to traffic with megalin on the cell surface (1,4). Mice in which the megalin gene has been “knocked out” excrete a variety of plasma proteins in increased quantities in their urine, which provides further evidence for the importance of megalin-dependent pathways for the recovery of low-molecular weight proteins and associated vitamins from the glomerular filtrate (6). Megalin and cubilin are recycled between apical clathrin-coated pits and early and late endosomes, with delivery of luminal ligands to the lysosomal compartment, where they are hydrolyzed to component amino acids. The details of this process remain to be elucidated. In addition, megalin was recently found to mediate protein transcytosis in an immortalized rat cell line (7).

As well as membrane-bound forms in tissues, both megalin and cubilin have been detected in soluble form in human urine (8–11). At least some of the soluble megalin is a truncated
form of that associated with the renal brush border membrane (10). Similarly, rat proximal tubule cells in culture may produce both membrane-bound and soluble megalin (12).

The renal Fanconi syndrome (Lignac-de Toni-Debré-Fanconi syndrome) consists of a generalized dysfunction of renal proximal tubules that leads, in its full form, to impaired proximal reabsorption of protein (tubular proteinuria), amino acids, glucose, phosphate, urate, and bicarbonate and rickets/osteomalacia (13). Dent’s disease (CLCN5 mutation) (14,15), the oculocerebrorenal syndrome of Lowe (OCRL1 mutation) (16), and autosomal dominant idiopathic Fanconi (ADIF) syndrome (17) are examples of renal Fanconi syndromes. Although other features of the Fanconi syndrome vary in these disorders, there is a consistent defect in renal tubular protein reabsorption (a process localized to the proximal tubules) (14). In Dent’s disease, in which a CLCN5 mutation leads to a defective CLC-5 chloride channel, there is evidence of a failure to acidify part of the endosomal compartment of proximal tubule cells (18–21). Furthermore, reduced apical expression of megalin in the proximal tubules was recently demonstrated in a mouse model of Dent’s disease (18).

Defective recycling of megalin and cubilin receptors, whether attributable to the probable failure of endosomal acidification (as in Dent’s disease) or to other mechanisms, might be expected to substantially change the rate of receptor transport to the luminal membrane, from which urinary receptors are presumably derived. We examined this hypothesis by studying the urinary excretion of receptors in several forms of the renal Fanconi syndrome, including Dent’s disease, Lowe’s syndrome, and ADIF syndrome. With the use of a validated urine preparation method, megalin was observed to be almost absent from the urine of most patients with Dent’s disease or Lowe’s syndrome.

### Materials and Methods

#### Patients

A total of 13 affected male patients with Dent’s disease were studied. Each patient, unless otherwise stated, bore the CLCN5 mutation of the disease and exhibited characteristic clinical and laboratory features, including tubular proteinuria (22–24). Details of the CLCN5 mutations and references to the full clinical descriptions and laboratory findings for each patient are as follows: patients C/II/2 and C/III/2 (total CLCN5 deletion) (family 1) (22), patient F/II/1 (W279X mutation) (family 2) (22), a member of family 7,194 (splice-site mutation with deletion of codons 132 to 241) (family 3) (15), patient 4/96 (R34X mutation) (family 4) (25), patient 6/97 (346-amino acid deletion) (family 5) (26), two members of a family with a Ser244Leu mutation (family 6) (24), one patient with an entire CLCN5 deletion (family 7) (26), and one patient with a TGG to TAG nonsense mutation at codon 343 (family 8) (26). Three patients from a family with a GGG to GAG missense mutation at codon 506 (family 9) were also studied. Two members of that family have mild atypical Dent’s disease, and one of those atypical patients (patient V-4) was one of the three patients from family 9 studied here (15,27,28); the other two affected male subjects studied exhibit the typical features of Dent’s disease. The three patients with Lowe’s syndrome (all male) exhibit severe mental retardation, growth retardation, visual impairment, and other clinical features typical of the disease, as well as tubular proteinuria (13,16); two of the patients are brothers. The two patients

### Table 1. Urinary excretion of megalin and cubilin by patients with Dent’s disease, Lowe’s syndrome, or autosomal dominant idiopathic Fanconi syndrome, compared with normal individuals

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 42)</th>
<th>Dent’s Disease</th>
<th>Lowe’s Syndrome (n = 3)</th>
<th>Autosomal Dominant Idiopathic Fanconi Syndrome (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megalin (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>100</td>
<td>7</td>
<td>106</td>
<td>6</td>
</tr>
<tr>
<td>range</td>
<td>40 to 172</td>
<td>0 to 11</td>
<td>82 to 150</td>
<td>3 to 9</td>
</tr>
<tr>
<td>Cubilin (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>100</td>
<td>124</td>
<td>125</td>
<td>115</td>
</tr>
<tr>
<td>range</td>
<td>45 to 185</td>
<td>75 to 164</td>
<td>80 to 145</td>
<td>78 to 130</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>80 to 125</td>
<td>55 (34 to 101)</td>
<td>84 and 18,^c^ 179^d^</td>
<td>33 (26 to 37)</td>
</tr>
<tr>
<td>Retinol-binding protein excretion (mg/mmol creatinine)^e^</td>
<td>0.0085 ± 0.00045^f^</td>
<td>23.6 ± 5.3^f^</td>
<td>22.4 and 16.1,^c^ 0.0083^d^</td>
<td>85 (68 to 93)</td>
</tr>
</tbody>
</table>

a Urinary excretion of megalin and cubilin was quantified as the band intensity (as integrated pixels) for the main megalin or cubilin bands for normal or affected individuals, expressed as a percentage of the integrated pixel (IP) value for a normal reference preparation of urine included on each gel [(IP-Patient/IP-Reference) × 100]. Each result was calculated as the mean of determinations on duplicate gels.

b Family 9 includes patients with both typical and atypical features of Dent’s disease (28).

c Two patients with typical Dent’s disease.

d Patient V-4, with atypical Dent’s disease, lacks tubular proteinuria but bears a CLCN5 mutation. Individual patients are described in the Materials and Methods section.

e Determined as an indicator of tubular proteinuria (44).

f Mean ± SEM.
with ADIF syndrome are father and son from the family that was originally reported (17). Table 1 presents measurements of creatinine clearance based on 24-h urine collections or calculated by using the Cockcroft-Gault method (29).

**Specimen Collection**

Midstream random urine specimens were collected without preservative, after at least 18 h of sexual inactivity. The samples were refrigerated immediately for up to 4 h and then either frozen at −80°C or in dry ice for up to 2 wk before transfer to liquid nitrogen for up to 1 yr before analysis. Specimens were thawed once only before analysis.

**Preparation of Urine**

A volume of well mixed urine equivalent to 25 μmol of creatinine was dialyzed (D9277 tubing; Sigma-Aldrich, Dorset, UK) for 4 h at 4°C, with vigorous stirring, against 3 L of 50 mM ammonium bicarbonate (Fluka 09830; Sigma-Aldrich), with one change after 1 h. This procedure removed >98% of the sodium and chloride from the urine. The dialysate was then lyophilized and stored at −80°C for up to 1 mo until analysis. Urine samples dialyzed and lyophilized in this way demonstrated good recovery of megalin and cubilin. In contrast, ultrafiltration concentrators could not be used to process urine; these produced large losses of the megalin present in normal urine, when normal urine was mixed with the megalin-deficient Fanconi syndrome urine and then concentrated by ultrafiltration. Addition of protease inhibitors did not abolish these losses (data not shown).

**Urine Ultracentrifugation**

Well mixed urine, to which protease inhibitors (P8340; Sigma-Aldrich) had been added at a concentration of 20 μg/ml urine, was centrifuged at 105 × g for 1 h at 4°C, in 1.5-ml aliquots. Supernatants were pooled and processed by rapid dialysis and lyophilization as described above. The pellets were resuspended in the same volume of 50 mM ammonium bicarbonate as the original urine samples and were dialyzed and lyophilized as described above.

**Preparation of Kidney Homogenates**

Normal human kidney cortex (200 mg wet weight) obtained during partial nephrectomy was homogenized on ice, with a ground-glass homogenizer, in 0.8 ml of 62.5 mM Tris-HCl (pH 6.8) containing protease inhibitors (20 μg/ml, P8340; Sigma-Aldrich).

**Megalin and Cubilin**

Megalin and cubilin, purified as described (30,31), and prestained molecular weight markers (C3312; Sigma-Aldrich) were used as standards for gel electrophoresis.

**Sodium Dodecyl Sulfate-Gel Electrophoresis and Blotting**

Urine lyophilizates were redissolved in 0.5 ml of 62.5 mM Tris-HCl (pH 6.8) with 5% sodium dodecyl sulfate (SDS), 10% glycerol, and 0.003% bromphenol blue and were heated at 40°C for 30 min. Twenty-five microliters were applied to 5% polyacrylamide gels (161-1210; Bio-Rad, Hertfordshire, UK) and subjected to electrophoresis in 25 mM Tris, 0.192 M glycine, at 200 V for approximately 35 min. Proteins were transferred to polyvinylidene difluoride membranes (Immobilon-P; Millipore, Hertfordshire, UK) in 25 mM Tris, 0.192 M glycine, at 150 V (limited to 35-W maximum power). Blots were blocked with 3% bovine albumin (A7638; Sigma-Aldrich) in 20 mM Tris-HCl, 0.5 M sodium chloride, 5 mM calcium chloride (pH 7.5) (TBSCa buffer), at room temperature for 2 h and at 4°C overnight. For kidneys, 1 ml of 62.5 mM Tris-HCl (pH 6.8) with 10% SDS, 20% glycerol, and 0.006% bromphenol blue was added to the homogenate from 200 mg of kidney, prepared as described above, with incubation as for urine; an additional 10-fold dilution with 62.5 mM Tris-HCl (pH 6.8) with 5% SDS, 20% glycerol, and 0.006% bromphenol blue was made before electrophoresis.

**Immunoblotting**

After blocking, membranes were rinsed in TTBSca buffer (TBSCa buffer containing 0.1% Tween 20) and probed by using one of the following protocols: (J) receptor-associated protein (RAP); 0.4 μg/ml rat RAP (62221; Progen Biotechnik, Heidelberg Germany) for 2 h, a 1:100 dilution of rabbit anti-human RAP (61098; Progen Biotechnik) (32) for 1 h, washing, and then a 1:40,000 dilution of anti-rabbit F(ab)2 conjugated to horseradish peroxidase (HRP) (NA9340; Amersham-Pharmacia Biotech, Buckinghamshire, UK); (β) anti-megalin: a 1:20,000 dilution of sheep anti-rat megalin (33) and then a 1:60,000 dilution of anti-sheep F(ab) conjugated to HRP (1301977; Roche Diagnostics, East Sussex, UK); (γ) anti-cubilin: a 1:40,000 dilution of rabbit anti-human cubilin (33) containing 0.2 mg/ml human IgG (14506; Sigma-Aldrich) and then a 1:40,000 dilution of anti-rabbit F(ab)2 conjugated to HRP; (δ) anti-cytoplasmic tail of megalin: a 1:20,000 dilution of a rabbit antibody to the cytoplasmic tail of megalin (designated 459) (34) and then a 1:40,000 dilution of anti-rabbit F(ab)2 conjugated to HRP. RAP and antibodies were prepared in TTBSca buffer with 1 mg/ml bovine albumin. Washes between steps were performed with TTBSca buffer. Final development was with ECL+ reagent (Amersham-Pharmacia Biotech, Buckinghamshire, UK).

**Chemiluminescence Recording**

Film (Bio-Max Light; Kodak, Hertfordshire, UK) was preflashed before exposure, and signals from scanned images (Epson GT-9500 Image Scanner with EU-14 transmission mode unit; Epson UK, Hertfordshire, UK) were processed with SigmaGel software (SPSS Inc., Chicago, IL) to yield an integrated pixel count for each band. Relative receptor excretion was then calculated as described in the footnote to Table 1 and the legend to Figure 2.

**Results**

When electrobtoths of SDS-polyacrylamide gels containing normal urine samples were probed with RAP, the binding pattern was dominated by a single band, which comigrated with authentic 600-kD megalin (Figure 1A, lanes 5 and 6). RAP is a high-affinity, chaperone-like ligand for megalin (10). Using the RAP binding system, we compared normal urine samples with urine specimens from patients with Dent’s disease and the three patients with Lowe’s disease and the three patients with Lowe’s syndrome but not in the one study patient with ADIF syndrome. To corroborate these results, we also detected the receptor by using a polyclonal antibody to megalin, instead of RAP binding. As indicated in Figure 1B, identical results were obtained by using anti-megalin antibodies, compared with RAP binding. The results presented in Figure 1...
were confirmed with at least one more urine sample from each patient, obtained several weeks later.

To explore this finding further, we examined urine specimens from 11 more patients with Dent’s disease and one additional patient with ADIF syndrome. The quantified normalized data for megalin excretion from all patients studied are presented in Figure 2 and Table 1. These data demonstrated almost complete deficiency of total urinary megalin for 10 patients with Dent’s disease, representing eight of the nine families studied. All three patients with Lowe’s syndrome were deficient in total urinary megalin. For the two patients with ADIF syndrome, no deficiency of either total or soluble megalin was observed (Figure 2 and Table 1). None of the normal individuals whose urine was examined exhibited results in the same range as values for patients with Dent’s disease or Lowe’s syndrome with megalin deficiency (Figure 2 and Table 1); therefore, the findings for these patients with Fanconi syndrome are unlikely to be attributable to normal variation. Among patients with Dent’s disease (families 1 to 8), Lowe’s syndrome (n = 3), and ADIF syndrome (n = 2), band intensities (as integrated pixels) for the main megalin or cubilin bands for patients or normal individuals were expressed as a percentage of the integrated pixel (IP) value for a normal reference preparation of urine included on each gel [(IPpatient/IPReference) × 100]. Each data point represents the mean of determinations on duplicate gels. Table 1 and the Materials and Methods section provide details for each patient group.

Cubilin is an endocytic receptor that exhibits ligand-binding properties distinct from those of megalin but may interact with megalin as a coreceptor (35,36). We therefore examined nor-
protein molecular masses of omission of anti-RAP. No significant bands corresponding to incubation with nonimmune rabbit serum in place of anti-RAP, or immunoblots were examined with omission of RAP, incubation with nonimmune rabbit serum in place of anti-RAP, or omission of anti-RAP. No significant bands corresponding to protein molecular masses of >180 kD were detected with either normal urine or urine from a patient with Fanconi syndrome. Similarly, use of nonimmune serum in place of the first antibody or omission of the first antibody in the anti-cubilin system did not generate significant bands (data not shown).

To detect possible urine-induced proteolysis in the samples from patients with Dent’s disease or Lowe’s syndrome, we examined the stability of the receptors megalin and cubilin in urine, by incubating together normal urine and megalin-deficient urine from patients with Dent’s disease, for up to 6 h at 37°C. No significant loss of megalin or cubilin was observed when the dialysis and lyophilization protocol described in the Materials and Methods section was used to process urine. In contrast, use of ultrafiltration concentrators led to losses of these receptors (data not shown).

The standard method used for the preparation of receptors from urine was specifically intended to include all species of receptors, i.e., those that might be bound to cell membranes or adsorbed onto particulate material as well as unbound forms. The results obtained with this method are considered to represent total urinary megalin levels. In addition, we determined whether there were soluble forms of receptors in the urine of these patients, as well as forms that could be sedimented by ultracentrifugation. Using the ultracentrifugation technique described in the Materials and Methods section, we analyzed urine samples by immunoblotting. In normal urine, little megalin or cubilin could be sedimented by ultracentrifugation (Figure 4c). Similar results were observed for one other member of family 9 with typical Dent’s disease and also a family member (patient V-4) with atypical disease (data not shown). Negligible amounts of megalin could be sedimented by ultracentrifugation from the urine of the two patients with ADIF syndrome (data not shown).

To further characterize the megalin excreted in urine, we examined whether the megalin in normal urine would react with the polyclonal antibody (antibody 459) (34) raised against a sequence in the cytoplasmic tail of megalin. Using this anti-cytoplasmic tail antibody, we observed no reactivity in a preparation from normal urine, under conditions in which a strong signal was observed in samples of human whole-kidney homogenate. The megalin antibody that had been raised against whole megalin yielded strong signals in both normal urine preparations and human kidney homogenates (as controls). Similarly, there was no reactivity of antibody 459 with the form of megalin that was sedimented by ultracentrifugation of urine from patients with Dent’s disease in family 9 (data not shown).

Discussion

These results are consistent with the involvement of defective trafficking of megalin in the pathogenesis of two major forms of the renal Fanconi syndrome, i.e., Dent’s disease and Lowe’s syndrome (15,37), and are also consistent with the recent finding of reduced apical expression of megalin in the renal proximal tubules in a mouse model of Dent’s disease (18). We suggest that failure to traffic megalin to the apical epithelium in proximal tubules, as part of normal recycling, results in a marked decrease in the loss of megalin into luminal fluid and thus into urine (Figure 5). In vitro studies previously suggested a central role for megalin in the pathogenesis of this disease (20,21).

A large body of work has established megalin as a major endocytic receptor in proximal tubules that binds several filtered proteins, including β2-microglobulin, retinol-binding protein, vitamin D-binding protein, and β2-glycoprotein I (1,6,38). Cubilin, in association with megalin, has recently
been observed to be an albumin receptor in the kidney (35,36), in addition to its previously identified role as a receptor for vitamin B$_{12}$-intrinsic factor in the ileum (39). Megalin is also thought to have roles in Ca$^{2+}$ transport and sensing, as well as signaling to the cell cytoplasm (40,41). Disruption of normal megalin trafficking would therefore be expected to interfere with both endocytic and signaling functions.

It is unclear why family 9 differs from the eight other families with Dent’s disease in excreting significant amounts of urinary megalin (Figure 2 and Table 1). Furthermore, unlike normal urinary megalin, the megalin in the urine of patients from this family occurs in a form that is sedimented by ultracentrifugation (Figure 4). This family is also unusual in that two male patients with a $CLCN5$ mutation have very mild, atypical disease, with either undetectable or slight tubular proteinuria, although the same mutation causes severe disease and marked tubular proteinuria among other members of the same family (28). The mutation in this family is a missense mutation substituting glutamine for glycine at codon 506 and is predicted to disrupt charge distribution in the highly conserved 11th transmembrane domain of the channel protein. When expressed in *Xenopus* oocytes, the mutant CLC-5 produces a chloride conductance that is indistinguishable from that in uninjected control cells and is indistinguishable from that produced by other mutations (missense, nonsense, and other mutations), represented in this study by patients with absent urinary megalin (15). We speculate that different $CLCN5$ mutations can have different effects on CLC-5, which are associated with the shedding of distinct forms of megalin into the lumen of the proximal tubules and thence into urine.

**Figure 4.** Forms of megalin and cubilin in urine. (a and b) Megalin (a) and cubilin (b) are present mostly in soluble form in normal urine. When normal urine was ultracentrifuged at 10$^5 \times g$ for 1 h, as described in the Materials and Methods section, essentially all of the receptor present in the original urine sample (lane 2) was recovered in the supernatant (lane 4), with almost none in the pellet (lane 3). Authentic megalin and cubilin are shown in lane 1. The positions of native megalin (600 kD) and cubilin (460 kD) and a 220-kD molecular mass standard are indicated by thick arrows; the unlabeled arrow in b indicates a probable cubilin dimer (see Discussion). (c) Unlike findings for patients with Dent’s disease from families 1 to 8, megalin was not deficient in the urine of a patient from family 9 and, unlike that in normal urine, megalin in the original urine sample from an affected patient with Dent’s disease in family 9 (lane 1) was present in both the pellet (lane 2) and the supernatant (lane 3) after ultracentrifugation at 10$^5 \times g$ for 1 h.

**Figure 5.** Model of megalin trafficking in a proximal tubule cell, to account for the decreased loss of megalin into urine for patients with the renal Fanconi syndrome attributable to Dent’s disease. Normal acidification of the sorting endosomes requires activity of both the vacuolar H$^+$-ATPase (H$^+$/H$_{ATPase}$) and the CLC-5 chloride channel (Cl$^-$/CLC-5), and there is failure of vesicle acidification in Dent’s disease because of the defective CLC-5 chloride channel. Normal recycling of megalin to the cell surface and physiologic release of the receptor therefore does not occur (see Discussion). Details of other known members of the endocytic apparatus, such as AP-2, are omitted. It is unclear whether acidification facilitated by CLC-5 has a direct effect on ligand dissociation from the receptor, as shown here, or an indirect effect attributable to failure to recruit endosomal regulatory proteins, as recently proposed by Maranda *et al.* (45).
Findings for the family with ADIF syndrome indicate that the urinary megalin deficiency observed in Dent’s disease and Lowe’s syndrome is specific and not secondary to the presence of other material such as proteases, which might be excreted in increased concentrations as part of the tubular proteinuria of the Fanconi syndrome. Furthermore, if the deficiency of megalin were attributable to the presence of proteases, then prolonged in vitro coinubcation of normal urine containing megalin with urine from patients lacking urinary megalin should have resulted in megalin losses. This was not observed, which further suggests that the observed deficiency of urinary megalin is not due to proteolytic or other events after shedding into the tubular lumen.

Lowe’s syndrome is attributable to mutation of the OCLR1 gene, which encodes a phosphatidylinositol-4,5-bisphosphate phosphatase (37). The results presented here implicate abnormal megalin receptor-mediated endocytosis in Lowe’s syndrome. Phosphatidylinositol-4,5-bisphosphate is localized primarily on the cytoplasmic face of the plasma membrane, and a role in the assembly of endocytic clathrin-coated vesicles has been identified (42). However, a link between the phosphatidylinositol-4,5-bisphosphate phosphatase defect and the possible endocytic defect in this syndrome remains to be described.

Results for the patients with ADIF syndrome suggest that megalin is delivered to the apical surface of the cell and may enter the tubular lumen at a relatively normal rate in this condition. A better understanding of the results for ADIF syndrome will require identification of the underlying mutation in this particular form of the Fanconi syndrome (17).

We have demonstrated megalin deficiency in the urine of several patients with normal levels of cubilin. This indicates that the megalin deficiency does not represent a global loss of proximal tubule receptors. The reason for normal urinary levels of cubilin, which is a coreceptor with megalin, is not known but might be related to the fact that cubilin apparently follows a different pathway during posttranslational processing, compared with megalin (43). The very slowly migrating form of cubilin observed in urine (Figures 3 and 4b) may correspond to the previously described disulfide-linked dimer (31,39).

The finding that negligible amounts of megalin and cubilin in normal urine can be sedimented by ultracentrifugation at 10^5 × g for 1 h suggests that these proteins are present in soluble form. This could be attributable to either release of the megalin ectodomain directly from the apical epithelium or degradation after release of the intact molecule in a membrane fragment, and our findings cannot differentiate between these possibilities. The mechanism by which soluble megalin is released from immortalized rat proximal tubule and rat yolk sac carcinoma cell lines may parallel the mechanism of megalin loss from the tubular apical epithelium in vivo (12). Lack of reactivity of urinary megalin with the anti-cytoplasmic tail antibody is consistent with the aforementioned finding that this megalin is soluble, suggesting that urinary megalin is generated by proteolytic activity. Further studies are needed to define the exact structures of urinary megalin.

This study presents the first human data that implicate abnormal function of the endocytic receptor megalin (gp330) in two major forms of the renal Fanconi syndrome, i.e., Dent’s disease and Lowe’s syndrome. This complements recent studies of ligand binding and cellular and animal models. Figure 5 presents a model to account for our findings for patients with Dent’s disease. On the basis of these studies, it may be possible to use the detection of megalin in urine as a functional assay to diagnose the failure or disruption of endocytosis and trafficking in renal proximal tubules.

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