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**Evaluation of the proximal tubular function in hereditary renal Fanconi syndrome**

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**Introduction**

The study of the proximal tubular function in inherited renal Fanconi syndrome offers a unique opportunity to augment insights in proximal tubular transport and signalling pathways and allows a better interpretation of tests applied for the evaluation of proximal tubular function in acquired disorders. The causes of inherited and acquired renal Fanconi syndrome are listed in Table 1. It is a common practice to evaluate proximal tubular function by measuring the re-absorption of low-molecular-weight proteins (LMWP) and the excretion of lysosomal enzymes. New information became available due to extensive studies performed in Dent’s disease, nephropathic cystinosis, Lowe syndrome and idiopathic Fanconi syndrome. Here we focus on the results obtained in these disorders.

**Molecular defects in Dent’s disease, nephropathic cystinosis and Lowe syndrome**

**Dent’s disease**

Dent’s disease is an X-linked proximal tubulopathy with Fanconi syndrome. Low-molecular-weight proteinuria is almost always present. Nephrocalcinosis and renal stone formation occur frequently. The disease is caused by molecular defects in the *CLCN5* gene (Xp11.22), encoding the voltage-gated chloride channel (CLC-5) [1]. Evidence for the role of CLC-5 in the acidification of endosomes was provided by several investigators. So endocytotic vesicles of CLC-5 knockout mice, compared to those of wild-type mice, displayed a slower acidification rate and a lower steady state of acidification after stimulation by ATP [2].

Two groups of investigators [3,4], however, demonstrated that CLC-5 functions as voltage-dependent electrogenic chloride/proton exchanger. This antiport would lead to a loss of accumulating protons, constituting a proton leak, which appears contrary to the original proposal that CLC-5 promotes endosomal acidification. Both groups of investigators suggested explanations for these contrasting findings. One suggestion is that CLC-5 might directly acidify early endosomes shortly after they punch off from the plasma membrane. Endosomal chloride at high extracellular concentration might exchange for cytosolic H⁺, resulting in acidifying of early endosomes [4].

**Cystinosis**

Cystinosis is an autosomal recessive disorder due to defective lysosomal cystine transport leading to accumulation of cystine in the lysosome. The disease is caused by mutations in the *CTNS* gene (17p13.3) encoding cystine-proton cotransporter cystinosin in the lysosomal membrane [5]. Earlier studies with cystine dimethylester (CDME), loading lysosomes with cystine, demonstrated a decrease of ATP production in proximal tubular cells pointing to ATP depletion being the underlying pathogenic mechanism in this disorder [6]. However, this attractive hypothesis was
Table 1. Causes of renal Fanconi syndrome

<table>
<thead>
<tr>
<th>Inherited Disorder/OMIM</th>
<th>Gene/inheritance</th>
<th>Protein</th>
<th>Key clinical/biochemical features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystinosis/Infantile 219800</td>
<td>CTNS/AR</td>
<td>Cystinosin</td>
<td>Failure to thrive, rickets, metabolic, acidosis, renal failure, photophobia</td>
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<tr>
<td>Juvenile 219900</td>
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<tr>
<td>Ocular 219750</td>
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<td></td>
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<tr>
<td>Dent’s disease/300009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CLCN5/XL-R</td>
<td>Chloride channel 5</td>
<td>LMW proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, renal failure</td>
</tr>
<tr>
<td>Lowe syndrome/309000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>OCRL1/XL-R</td>
<td>Phosphatidyl-inositol 4,5-biphosphate-5-phosphatase</td>
<td>Short stature, congenital cataract, mental retardation, seizures, cryptorchidism, elevated transaminases</td>
</tr>
<tr>
<td>Hereditary fructose intolerance/229600</td>
<td>ALDOB/AR</td>
<td>Aldose B</td>
<td>Fructose intolerance, growth retardation</td>
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<tr>
<td>Galactosaemia/230400</td>
<td>GALT/AR</td>
<td>Gal-1-P uridyl-transferase</td>
<td>Hepatomegaly, liver disease, cataract, mental retardation</td>
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<tr>
<td>Tyrosinaemia/276700</td>
<td>FAH/AR</td>
<td>Fumaryl-acetoacetate hydrolase</td>
<td>Glomerulosclerosis, nephrocalcinosis, hepatomegaly, cirrhosis, rickets, growth retardation</td>
</tr>
<tr>
<td>Wilson disease/277900</td>
<td>ATP7B/AR</td>
<td>Copper-transporting ATPase, β Polypeptide</td>
<td>Kayser–Fleischer rings (cornea) hepatitis, cirrhosis CNS abnormalities</td>
</tr>
<tr>
<td>Fanconi–Bickel syndrome/227810</td>
<td>SLC2A2/AR</td>
<td>GLUT2</td>
<td>Hepatorenal glycogen accumulation, hepatomegaly, rachitic and osteomalacia, mental retardation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Acquired Mitochondrial disorders</th>
<th>Diverse</th>
<th>Diverse</th>
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<tbody>
<tr>
<td>Intrinisc renal disease</td>
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<tr>
<td>Acute tubular necrosis</td>
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<td>Myeloma</td>
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<td>Sjögren syndrome</td>
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<td>Transplant rejection</td>
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<td>Pharmaceuticals</td>
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<tr>
<td>Cisplatin</td>
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<td>Ifosfamide</td>
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<td>Gentamicin</td>
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<td>Valproic acid</td>
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<td>6-mercaptopurine</td>
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<td>Other exogenous toxins</td>
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<tr>
<td>Glue sniffing</td>
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<td>Heavy metals (mercury, lead, cadmium, uranium)</td>
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<td>Maleic acid (in experimental animals)</td>
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<tr>
<td>Nutritional Kwashiorkor</td>
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AR, autosomal recessive; XL-R, X-linked recessive; CNS, central nervous system.

<sup>a</sup>Gluicosuria can be absent.

not confirmed in cystinotic fibroblasts demonstrating normal ATP-generating capacity [7]. Recently, it was demonstrated that CDME had a direct toxic effect on mitochondrial ATP production and cell viability, which was not observed in cystinotic cells with comparable cystine levels [8]. Further investigations in cystinotic proximal tubular cells and a mice model of cystinosis will solve the question whether ATP metabolism is disturbed in cystinosis. Increased apoptosis and altered metabolism of glutathione were demonstrated in vitro in cystinotic cells [9–11], however, it remains obscure, how these mechanisms are linked to lysosomal cystine accumulation. Recent study showed that the expression of cystinosin splice variant, lacking carboxy terminal lysosomal targeting motif, is not restricted to the lysosome and is also present on the plasma membrane, in the endoplasmatic reticulum, the Golgi apparatus and small cytosolic vesicles. This intriguing finding suggests that this protein has yet unknown functions within the cell, which can be relevant for understanding the pathogenesis of cystinosis [12].

**Lowe syndrome**

Oculocerebrorenal syndrome of Lowe (OCRL) is an X-linked disorder characterized by congenital cataracts, mostly incomplete renal Fanconi syndrome and mental
Role of megalin/cubulin in the reabsorption of LMWP by the proximal tubule

LMWP are reabsorbed by endocytosis via the multiligand tandem receptors megalin and cubulin expressed at the brush border of the proximal tubule [20,21]. 

Megalin knockout (KO) mice exhibit a tubular reabsorption defect with an increased excretion of LMWP. As the plasma concentration of LMWP and the GFR were not measured in these mice, it is unclear whether megalin/cubulin forms the unique pathway for reabsorption of LMWP. No significant differences between knock-out urine samples compared to controls were observed for glucose, electrolytes, urea, uric acid and amino acids [22,23].

In an experimental model for Dent's disease (CLC-5 KO mice), Christensen et al. demonstrated a redistribution of megalin and cubulin from the brush border to intracellular endosomes suggesting a slowing down of the recycling of megalin and cubulin to the brush-border membrane [24]. Norden et al. found in dialyzed and lyophilized urine samples in eight of nine families with Dent's disease and two families with Lowe syndrome but not in autosomal idiopathic Fanconi syndrome a deficiency of urinary megalin compared with normal controls, consistent with a reduced apical expression of megalin [25]. It was suggested that urinary megalin was generated by proteolytic activity because it did not react with an antibody directed to the cytoplasmic tail of the protein. In some, but not all, studied patients with Dent's disease, a decreased expression of megalin in the brush border of the proximal tubule can be found by an immunofluorescence technique [26–28].

The role of megalin/cubulin in other inherited disorders with LMWP has to be investigated.

Lysosomal enzymuria

In a recent paper, Norden et al. reported an increased excretion of both cathepsin D (MW 48 kDa) and N-acetyl-β-glucosaminidase (MW 130 kDa) in patients with Dent's disease, Lowe syndrome, cystinosis and autosomal dominant idiopathic Fanconi syndrome [29]. Urinary cathepsin D had a mannose-6-phosphate tag. This tag attachment is the first step for the transport of diverse lysosomal hydrolases from the Golgi apparatus to the late endosomes. Necrosis of the proximal tubular cells as basis for the origin of both hydrolases was excluded as the urinary lactate dehydrogenase (LDH) concentrations were normal in these patients. Based on the calculated glomerular protein sieving coefficient, a tubular origin of cathepsin D was suggested. The glomerular sieving coefficients were calculated assuming that glomerular filtration is the only source of a protein in urine of the patients with Fanconi syndrome without losses or additions to the filtrate. The authors propose a trafficking defect delivering lysosomal enzymes to the apical membrane by a default pathway. The exact mechanism, however, remains obscure.

In mouse kidneys with defective megalin expression (megalin KO and CLC-5 KO) a high excretion of another lysosomal hydrolase cathepsin B (MW 31 kDa) lacking mannose-6-phosphate was caused by impaired reabsorption of the enzymes [30]. A direct interaction of cathepsin B with megalin was demonstrated by surface plasmon resonance. Contrasting the findings of Norden et al. in patients with renal Fanconi syndrome, urinary cathepsin B contained no detectable mannose-6-phosphate arguing against a primary defect in intracellular sorting. Both mechanisms described by Norden et al. and Nielsen et al. can have a role in lysosomal enzymuria in human Fanconi syndrome.

Conclusion

The decrease in the reabsorption of LMWP and lysosomal hydrolases in Dent's disease and Lowe syndrome can be probably attributed to the decreased presence of megalin/cubulin at the brush border membrane. However, lack of megalin cannot explain other signs of proximal tubular dysfunction such as aminoaciduria. Renal expression of megalin and cubulin still has to be investigated in other inherited renal Fanconi syndromes.

A role of an altered megalin/cubulin expression in acquired disorders is not known. Histologically proven tubulo-interstitial abnormalities are considered to be the cause in most disorders with an increased excretion of LMWP and lysosomal hydrolases. Interestingly, in steroid-sensitive nephrotic syndrome without histological evidence of tubulo-interstitial involvement, the urinary excretion of LMWP and acetyl-β-D-glucosaminidase can also be increased [31]. The excretion of the most LMWP significantly correlates with albumin excretion. A decreased
megalin-mediated uptake due to competition from increased protein load offers an attractive hypothesis for increased LMWP excretion in acquired disorders.

Conflict of interest statement None declared.

(See related article by A. G. W. Norden et al. Lysosomal enzymuria is a feature of hereditary Fanconi syndrome and is related to elevated CI-mannose-6-P-receptor excretion. Nephrol Dial Transplant 2008; 23: 2795–2803.)

References


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