Molecular developments in renal tubulopathies

The renal tubule is responsible for the reabsorption of more than 99% of the water and sodium in the glomerular filtrate. Congenital or acquired tubular dysfunction can therefore readily cause profound electrolyte and volume disturbance. The tubule also has to regulate acid-base balance, mineral homoeostasis, and the excretion of organic anions and drugs. To fulfil these functions, a large number of specialised transporters and channels are specifically localised in the tubular cell membranes, some in the luminal border and others in the plasma membrane border (basolateral membrane). In the past decade (and especially in the past five years) advances in molecular genetic research have revealed the structure, function, and effects of mutations in these transporters, thereby greatly increasing our understanding of the function and dysfunction of the renal tubule. Some renal stone disorders (for example, cystinuria, Dent's disease) and rare genetic causes of hypertension (for example, Liddle's syndrome) are now known to be caused by mutations in tubular transport systems.

Presentation
Many children with genetic defects in tubular function present in infancy although there are several, less severe disorders that present later or may be asymptomatic (for example, Gitelman's syndrome) and may only be detected when the patient has a blood or urine sample taken as part of a routine assessment. As a result the true incidence of some of these defects is not known. Many tubulopathies lead to failure to thrive; those causing chronic dehydration, salt wasting, or acidosis will inevitably impair growth, while excessive phosphate wastage will lead to rickets and retard bone development. Children with renal stones or who are found to have nephrocalcinosis require investigation of their tubular function.

The initial assessment of tubular function is based on the results of routine biochemical investigations. The request for plasma biochemistry should include sodium, potassium, urea, creatinine, bicarbonate, chloride, calcium, phosphate, and magnesium, and a urine sample should be collected for determination of urine electrolytes (sodium, potassium, chloride, calcium, phosphate), amino acids, glucose, and creatinine. Documentation of urine pH (by glass electrode) during an episode of severe acidosis (either spontaneous or induced by ammonium chloride) helps to differentiate between proximal and distal renal tubular acidosis (RTA). In proximal (type II) RTA, at a time of severe acidosis, the urine pH will fall to less than 5.5, while in distal (type I) RTA, the urine pH will remain above 6. The osmolality of a sample of the first morning urine is a useful check of renal concentrating ability (normally more than 800 mOsm/kg, but less in infants). Determination of urinary low molecular weight proteins (for example, retinol binding protein, β2microglobulin) and enzymes (for example, N-acetyl glucosaminidase) provides more sensitive and specific evidence of proximal tubular dysfunction and/or damage. An abdominal ultrasound to check the renal anatomy and to look for the presence of nephrocalcinosis, together with a radiograph of the wrists or knees for evidence of rickets, completes the basic assessment.

The proximal renal tubule is the site of the bulk of solute and water reabsorption in the nephron. Approximately 60% of the filtered sodium is reabsorbed in the proximal segments, along with water, potassium, bicarbonate, phosphate, amino acids, and low molecular weight proteins. Dysfunction of the proximal tubule may be isolated or generalised. In contrast, the distal tubule has a specialised role in the final modification of urine. Specialised transporters are involved in the regulation of sodium and potassium reabsorption and in proton secretion. Disorders of the distal tubule therefore tend to be isolated to a specific transporter.

Proximal tubulopathies
Proximal RTA in children is usually seen as a part of the renal Fanconi syndrome, consisting of aminoaciduria, glycosuria, bicarbonaturia, phosphaturia, and rickets. Most paediatric cases occur as part of a metabolic disorder although it may be found in some nephropathies and following exposure to some drugs and toxins (see table 1). A Fanconi like syndrome can be seen in severe vitamin D deficiency; similar plasma biochemical abnormalities can also be seen in patients with chronic diarrhea. Recent evidence supports the theory that there is a common pathogenetic mechanism for the Fanconi syndrome. Rather than there being a multiplicity of defective transporters accounting for the many solutes lost in excess, it is far more likely that the various metabolic disorders all, in some way, reduce the availability of ATP for the enzyme NaK-ATPase, thereby reducing sodium extrusion from the tubular cell and reducing the gradient for solute transport which is coupled to sodium reabsorption.1 Such generalised dysfunction often causes severe sodium and water losses in addition to the other electrolytes (potassium, bicarbonate, phosphate, etc.).

A better understanding of the mechanisms whereby these metabolic disorders affect energy production in the tubular cell should follow from knowledge of the molecular bases of these conditions. The gene for cystinosis has recently been isolated and characterised.2 Most affected children of European origin share a common 65 kb deletion (which should make molecular diagnosis viable). The gene (CTNS) codes for a protein, cystinosin, predicted to be located in the lysosomal membrane. This is consistent with the known biochemical basis of defective lysosomal cystine transport, but further functional work is needed to prove that cystinosin is indeed a cystine transporter. Dent's disease is an X linked condition in which affected males develop low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, generalised proximal tubular dysfunction with rickets, and in adult life, may suffer renal stones and chronic renal failure. The disorder is caused by mutations in a voltage gated chloride channel, ClC-5, expressed predominantly in the kidney in the subapical endosomes (precursors of lysosomes).3 As in cystinosis, disruption of intracellular organelle function (endosome or lysosome) leads to tubular cell dysfunction. Children affected with the Fanconi–Bickel syndrome present with hepatomegaly and failure to thrive. They have hepatic glycogenosis, fasting ketonuria, and hypoglycaemia (features of decreased mobilisation of glucose) and
Table 1  Inherited causes of the renal Fanconi syndrome

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Onset/Features</th>
<th>Defective gene/protein</th>
<th>Diagnostic test</th>
<th>Specific treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystinosis</td>
<td>Mid/late infancy, poor growth, may be blond/fair hair, corneal cystine crystals</td>
<td>CTNS/cystinosin</td>
<td>Leucocyte cystine concentration</td>
<td>Cysteamine</td>
</tr>
<tr>
<td>Tyrosinaemia</td>
<td>Infancy, poor growth, hepatic enlargement and dysfunction</td>
<td>Fumaryl acetoacetate hydrolase</td>
<td>Plasma amino acids, urine organic acids (succinyl acetone)</td>
<td>Nitro-trifluoro-benzyl cyclohexidine (NTBC)</td>
</tr>
<tr>
<td>Lowe's syndrome</td>
<td>Birth, X linked, cataracts, hypotonia, developmental delay</td>
<td>Inositol polyphosphate 5-phosphatase</td>
<td>Clinical and molecular genetic diagnosis</td>
<td></td>
</tr>
<tr>
<td>Galactosaemia</td>
<td>Birth, jaundice, encephalopathy</td>
<td>Galactose 1-phosphate uridylic transferase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructosemia</td>
<td>Rapid onset after fructose given, vomiting, hypoglycaemia, hepatomegaly</td>
<td>Fructose-1-phosphate aldolase B</td>
<td>Hepatic fructose-1-phosphate aldolase B</td>
<td>Fructose and sucrose free diet</td>
</tr>
<tr>
<td>Fanconi–Bickel syndrome</td>
<td>Infancy, failure to thrive, hepatomegaly, galactosuria</td>
<td>GLUT2/Glut2 (facilitated glucose transporter)</td>
<td></td>
<td>? Monosaccharide diet</td>
</tr>
<tr>
<td>Mitochondrial disorders</td>
<td>Usually in infancy, may be multisystem dysfunction</td>
<td>Mitochondrial DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wilson's disease</td>
<td>Childhood, hepatic disease, neurological signs, Kayser–Fleischer rings</td>
<td>IF-1/P-type copper transporting ATPase</td>
<td>Copper, caeruloplasmin</td>
<td>D-penicillamine</td>
</tr>
</tbody>
</table>

post-prandial hyperglycaemia, galactosaemia, and galactosuria (features of decreased utilisation of glucose and galactose). These features led workers to speculate that these children had a defect in monosaccharide transport and this has now been established. Patients have mutations in a gene encoding Glut2, one of four facilitated di-

and this has now been established. Patients have mutations in the PHEX gene which codes for a membrane glycoprotein that has endopeptidase activity (endopeptidases have an important regulatory effect on some hormones\(^5\)). Patients have mutations in the PHEX gene which codes for a membrane glycoprotein that has endopeptidase activity (endopeptidases have an important regulatory effect on some hormones\(^5\)). There are rarer disorders with hypophosphataemic rickets, inherited in an autosomal recessive form associated with hypercalciuria.

Barter's and Gitelman's syndromes
Since their description some 30 years ago, there has been much debate about the pathogenesis of Barter's syndrome and its variants.\(^7\) The presentation varies from polyhydranmios, prematurity, and life threatening neonatal electrolyte disturbance, through failure to thrive to asymptomatic detection of abnormal biochemistry in later life. These disorders share features of hypokalaemic alkalosis in the presence of a normal blood pressure, elevation of plasma renin and aldosterone, and increased urinary chloride and potassium loss. Some have hypercalciiura and nephrocalcinosis, some have hypomagnesaemia and hypocalciuria. Recent work has greatly illuminated our understanding of these disorders and thereby of electrolyte transport in the loop of Henle (see fig 1). Sodium chloride is absorbed in the thick ascending limb by a sodium–potassium-2 chloride cotransporter (NKCC2), the target for inhibition by loop diuretics (for example, frusemide). Reabsorption of sodium and chloride by NKCC2 depends in turn on basolateral NaK-ATPase (to generate a gradient for sodium entry into the cell), recycling of potassium out of the tubular cell into the lumen (using a potassium channel, ROMK), and transport of chloride across the basolateral membrane (by another chloride channel, CIC-Kb).

Mutations in the genes coding for NKCC2, ROMK, and CIC-Kb have been identified in patients with various Bartter syndromes and there is some correlation between the genotype and phenotype.\(^6^-^10\) In general, children with the more severe phenotype (neonatal Bartter syndrome) tend to have mutations in the genes coding for NKCC2 or ROMK while those with the milder "classic" Bartter's syndrome have mutations affecting the chloride channel CIC-Kb. Individuals with Gitelman's syndrome are often asymptomatic, but are characterised biochemically by hypokalaemic alkalosis, hypomagnesaemia, and hypocalciuria. The molecular defect affects another sodium chloride cotransporter (NCCT) but with differences to NKCC2. NCCT is localised in the distal tubule, does not transport potassium, and is the target for thiazide diuretics.\(^11\) The mechanism of hypomagnesaemia is not proven.

Distal renal tubular acidosis
Distal RTA is characterised by a failure of urinary acidification, hypokalaemia, hypercalciuria leading to nephrocalcinosis and, potentially, stone formation. In contrast to proximal RTA which in children is usually part of the generalised Fanconi syndrome, distal RTA commonly occurs as the only tubular abnormality. Various inheritance patterns are known. An autosomal dominant form, more obvious in adulthood, appears to be related to mutations in

![Figure 1 Schematic view of a renal tubular cell in the thick ascending limb of the loop of Henle.](http://adc.bmj.com)
an anion exchanger (AE1). AE1 (or band 3) is expressed in erythrocytes and in the basolateral membrane of the intercalated cells of the renal tubule. A significant number of patients with autosomal recessive distal RTA also have sensorineural deafness. The pathogenetic basis of this association has now been revealed by recent molecular studies. Urinary acidification in the distal tubule relies on secretion of hydrogen ions which is achieved by H\textsuperscript{+}/ATPases located in the apical membrane. Mutations in ATP6B1, the gene coding for the B1 subunit of apical by H\textsuperscript{+}/ATPases are found in patients with recessive distal RTA and would lead to failure of acid secretion. B1 subunit containing H\textsuperscript{+}/ATPases are also expressed in the inner ear epithelium so that it is possible that mutations in the gene coding for this unit could affect endolympathic acid–base balance at this site and consequently impair hearing.

Abnormalities of the amiloride sensitive epithelial sodium channel
Pseudohyposalpinost deronism type 1 (PHAI) is a recessive disorder of severe urinary salt wasting, presenting in the neonatal period with weight loss, vomiting, dehydration, and sometimes respiratory distress. It is characterised, biochemically, by hyponatraemia, severe hyperkalaemia, and raised plasma renin and aldosterone concentrations. Recent work has shown that PHAI results from mutations in the three subunits of the amiloride sensitive epithelial sodium channel. Other mutations in subunits of the same sodium channel lead to a rare autosomal dominant form of hypertension (Liddle’s syndrome). Thus some mutations cause gain of function, increased sodium–potassium exchange in the distal tubule, and hypertension (Liddle’s) whereas loss of function mutations are associated with salt loss (PHAI).

Nephrogenic diabetes insipidus
Congenital nephrogenic diabetes insipidus (NDI) is usually inherited in an X linked manner and affected males typically present in the newborn period with severe polyuria and polydipsia, poor sleep, recurrent vomiting, and constipation. Plasma biochemistry shows hypernatraemia and the urinary sodium is low; plasma arginine vasopressin and plasma osmolality are abnormally raised while the corresponding urine values are inappropriately low. Administration of DDAVP fails to correct the urine concentrating defect. The disorder arises as a result of mutations in the gene encoding the vasopressin receptor in the collecting duct cells (V2R). These prevent vasopressin binding to the receptor or inhibit the signal transduction which normally leads to distribution of aquaporin-2 (AQP2) water channels to the apical membrane of the tubular cell. A rare autosomal recessive form of NDI is a result of mutations in the AQP2 gene.

Conclusions
The recent explosion in studies defining the molecular basis of disease has greatly increased our knowledge of the renal tubule and has defined the pathogenesis of many tubulopathies. There is still much to be done and atypical cases (for example, Barter’s syndrome variants) will continue to pose challenges. These genetic studies now need to be followed by a return to biochemical research to link the mutation to the phenotype. This is especially relevant in proximal tubular disorders where an insight to intracellular dysfunction in single gene disorders is likely to reveal the mechanisms of the commoner acquired tubular dysfunction (for example, in acute tubular necrosis). In addition, funding for research initiatives has not yet been paralleled by provision of routine molecular genetic diagnostic services. The diagnosis of a renal tubulopathy therefore continues to rely on a high index of suspicion and the correct interpretation of plasma and urine biochemical data.

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