Mini-review

Straightening out the renal tubule: advances in the molecular basis of the inherited tubulopathies

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Introduction

The cell membranes of the renal tubular epithelium contain many specialized channels and transporters, which allow the regulation of the volume and solute content of the body by the selective reabsorption or secretion of different ions into the urine. In recent years, investigation into the pathogenesis of the inherited renal tubulopathies (Table 1) has demonstrated abnormalities in a number of different ion channels and transporters, and such studies have provided some insight into the complex cellular mechanisms involved in urinary solute reabsorption. I review the clinical features and pathophysiology of five of these rare inherited tubulopathies, and discuss recent molecular advances, which help to clarify some aspects of the physiology of renal tubular functioning and the pharmacology of diuretic action.

Liddle’s syndrome

Liddle’s syndrome (pseudohyperaldosteronism) is characterized by hypertension, hypokalaemic metabolic alkalosis, and suppressed plasma renin and aldosterone levels.1,2 It is inherited as an autosomal dominant disorder. Affected members of Liddle’s families have a high risk of cerebral haemorrhage and cardiovascular disease.2 However, neither hypertension nor hypokalaemia are invariant features, and may be absent in some obligate carriers, although renin and aldosterone levels are subnormal.2 Hypertension is due to plasma volume expansion,3 and effective blood pressure control relies upon dietary sodium restriction.1−4 Several studies have demonstrated that the hypokalaemia of Liddle’s syndrome could be corrected by the combination of sodium restriction and administration of triamterene or amiloride, but not by the mineralocorticoid receptor antagonist, spironolactone.1−3 This suggested that the primary defect in the disorder was one of abnormal renal sodium reabsorption,1 and that this defect occurred at a point downstream of the mineralocorticoid receptor. Furthermore, the index case described by Liddle1 had poorly controlled hypertension and developed end-stage renal failure 27 years after initial diagnosis.2 Her metabolic abnormalities resolved following renal transplantation, and this provided further evidence that the primary defect in Liddle’s syndrome was localized to the kidney.2

The various pathophysiological features of Liddle’s syndrome suggested that the amiloride-sensitive epithelial sodium channel (ENaC), which mediates aldosterone- and vasopressin-responsive sodium reabsorption in the distal nephron could be abnormal in Liddle’s patients.2,5 This channel consists of three homologous subunits, which are encoded by three different genes: the αENaC gene which is located on the short arm of chromosome 12, and the β- and γ- ENaC genes which both map to a small interval on the short arm of chromosome 16.6,7 Complete linkage of the Liddle’s phenotype to genetic markers in the region of the β- and γ- ENaC genes on chromosome 16 was subsequently demonstrated.6,7 Mutational analysis of the βENaC gene in five kindreds showed heterozygous point mutations which were all clustered within a 95 bp region, and which predicted premature truncation of the intracellular carboxyl-terminal tail of the βENaC gene.
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<td>XR</td>
<td>LMMP, PCT, TAL, DCT</td>
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†, decreased; †, increased; N, normal; †, present; †, absent; U, unknown; AD, autosomal dominant; AR, autosomal recessive; XR, X-linked recessive; SS, short stature; MR, mental retardation; LMMP, low-molecular mass proteinuria; GI, renal glycosuria; PO₄, phosphaturia; AA, amino aciduria; mTAL, medullary thick ascending limb; PCT, proximal convoluted tubule; DCT, distal convoluted tubule; DT/CD, distal tubule/collecting duct.
protein (Figure 1). Further studies of the γENaC gene in a Liddle’s kindred who were found to have a normal βENaC gene, showed a similar mutation in the intracellular tail of γENaC.\textsuperscript{7}

The mechanism by which these truncations of βENaC and γENaC cause the excessive sodium reabsorption found in the Liddle’s phenotype (i.e. activation of ENaC function), however, was unclear.\textsuperscript{6,7} Subsequent expression studies have shown that these mutations lead to an increase in the number of ENaCs at the apical surface of the tubular cell, which would be expected to result in the increased sodium reabsorption observed.\textsuperscript{8} This increased renal sodium reabsorption in the distal nephron therefore directly explains the volume expansion and hypertension found in Liddle’s syndrome, whereas the hypokalaemic alkalosis is due to the functional coupling of potassium and hydrogen secretion to sodium reabsorption in this tubular segment.\textsuperscript{9}

**Pseudohypoaldosteronism**

Pseudohypoaldosteronism (type 1) (PHA1) is generally manifest within the first few weeks of life, with failure to thrive, hyponatraemia with renal salt wasting, dehydration, and a profound hyperkalaemic metabolic acidosis.\textsuperscript{10–12} Plasma renin and aldosterone levels are elevated, and the salt wasting is unresponsive to exogenously administered mineralocorticoids.\textsuperscript{10,12} Although most subjects present in infancy, some individuals may remain asymptomatic into adult life, and are detected by biochemical screening.\textsuperscript{12} PHA1 has been reported to occur as a sporadic condition, and as both an autosomal recessive and an autosomal dominant trait.\textsuperscript{10–12} However, the autosomal dominant form of the disorder may be asymptomatic, with biochemical evidence of activation of the renin-angiotensin-aldosterone axis, but with little salt wasting or dehydration.\textsuperscript{12} The disorder can be managed with dietary sodium

**Figure 1.** Schematic representation of the nephron, with the proposed topographical structures of the various ion transporters and channels implicated in inherited tubulopathies. The percentage of filtered sodium and calcium reabsorbed in each nephron segment is also shown. The nomenclature of the channel and transporter genes has been simplified throughout the text for clarity. The gene encoding CLC5 is also known as CLCN5. The NKCC2 and NCCT transporters are also referred to as hBSC and hTSC (human bumetanide- and thiazide-sensitive cotransporters). The NKCC2 gene has also been referred to as SLC12A1. The ROMK1 gene is also called KCNJ1, and the γ to γENaC genes are also called SCNNA, SCNNB and SCNNG. The CLC5 chloride channel is ubiquitously expressed along the length of nephron, but the Fanconi syndrome found in X-linked hypercalciuric nephrolithiasis suggests that abnormalities of CLC5 have a major effect in the proximal tubule. The hypercalciuria and possibly other features of X-linked hypercalciuric nephrolithiasis may be explained by CLC5 dysfunction in the mTAL or distal tubule.
supplementation in early childhood, but as salt wasting decreases with age, this can be often discontinued in later life.

Since PHA1 is associated with high levels of aldosterone and apparent resistance to mineralocorticoid action, molecular genetic investigations initially focused upon the mineralocorticoid receptor gene, which was found to be structurally normal in affected subjects. Subsequently, a genome-wide search in autosomal recessive PHA1 kindreds identified linkage to genetic markers on the short arms of chromosomes 12 and 16 in different families; the same regions where the α-, β- and γ-amiloride-sensitive epithelial sodium channel (ENaC) subunit genes had been previously mapped. Mutational analysis of the genes encoding all three ENaC subunits showed homozygous truncating mutations in the γENaC gene in four autosomal recessive PHA1 kindreds, and a homozygous missense mutation (amino acid substitution) in the βENaC gene in another kindred. Similar studies in another three autosomal recessive PHA1 families, all originating from the Indian subcontinent, revealed the same homozygous splice site mutation of the γENaC gene in each family. All of these mutations would be predicted to abolish or reduce ENaC function, and this has been confirmed for the missense mutation of the βENaC. Thus, autosomal recessive PHA1 is genetically heterogeneous, with inactivating mutations in all three ENaC subunit genes leading to the phenotype. Furthermore, the effects of a genetic inactivation of the ENaC, (i.e. salt wasting, dehydration, hyperkalaemia and acidosis), resemble closely the pharmacological actions of the potassium-sparing diuretics amiloride and triamterene on the ENaC channel in the distal tubule. It remains possible that other families with PHA1 (perhaps with autosomal dominant inheritance) have mutations either in another distal tubular channel or in the mineralocorticoid receptor gene.

Bartter’s syndrome

Bartter’s syndrome classically presents in infancy or early childhood with failure to thrive, polyuria, nephrocalcinosis, short stature and mental retardation. Biochemically, it is characterized by a hypokalaemic metabolic alkalosis with renal salt wasting, nephrogenic diabetes insipidus, and hypercalciuria. It is usually a sporadic condition, but may be inherited as an autosomal recessive trait, and is particularly common in African-American and Afro-Caribbean populations. The blood pressure is typically normal, but there is profound activation of the renin-angiotensin II-aldosterone axis, associated with the hyperplasia of the juxtaglomerular apparatus originally described by Bartter, and excessive intra-renal production of prostaglandin E2. While some subjects remain asymptomatic into adult life, there is a subgroup of severely affected children in whom features of the disorder are manifest before birth. Such children, who have been referred to as having the ‘antenatal variant’ of Bartter’s syndrome or the hyperprostaglandin E syndrome, present with polyhydramnios due to foetal polyuria and premature delivery often follows. After birth, this variant may be life-threatening with hypotension, polyuria, severe salt wasting and systemic symptoms such as fever, vomiting and diarrhoea, due to the high levels of circulating prostaglandin E2. Nephrocalcinosis may progress rapidly in such children, due to the severe hypercalciuria, and osteopaenia may also be present. The mainstays of treatment of Bartter’s syndrome are combinations of potassium supplements or potassium-sparing diuretics (e.g. amiloride), with indomethacin or angiotensin-converting enzyme inhibitors.

Recent studies into the molecular basis of Bartter’s syndrome have focused on the bumetanide-sensitive sodium-potassium-chloride cotransporter (NKCC2), which is responsible for ‘electroneutral’ sodium and chloride reabsorption through the apical membrane of the medullary thick ascending limb of the loop of Henle (mTAL). Loop diuretics are known to be specific antagonists of NKCC2, and their actions produce the familiar effects of kaliuresis, salt wasting, concentrating defects and hypercalciuria, which closely resemble the urinary abnormalities found in Bartter’s patients. Furthermore, it has recently been demonstrated that severely affected Bartter’s patients have an impaired diuretic response to furosemide, directly implicating NKCC2. The gene for NKCC2 encodes a protein with 12 transmembrane domains, and maps to the long arm of chromosome 15. Linkage studies demonstrated that affected children from four consanguineous Bartter’s families were all homozygous for genetic markers over this area of chromosome 15, and mutational analysis of the NKCC2 gene showed homozygous or compound heterozygous point mutations in each of nine affected subjects. These mutations were spread throughout the gene, and many of them predicted premature truncation of the NKCC2 protein, consistent with inactivation or reduction in function of the cotransporter.

Investigation of other families with Bartter’s syndrome has, however, shown that the situation is more complicated than originally thought, as genetic linkage studies were able to exclude the chromosome 15 (NKCC2) locus in 6/14 kindreds. Net sodium and chloride reabsorption occurs through the apical membrane of the mTAL by a close coupling of synchronous sodium, potassium and chloride uptake (with a stoichiometry of 1Na:1K:2Cl)
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through the electroneutral NKCC2 cotransporter, and concurrent potassium secretion (recirculation) through the ATP-regulated potassium channel, ROMK1, in the opposite direction (Figure 2). Thus, it seemed possible that a disruption to normal ROMK1 functioning would have similar effects on sodium and chloride uptake to abnormalities of NKCC2 function, which caused Bartter's syndrome. Mutational analysis of the ROMK1 gene demonstrated homozygous or compound heterozygous mutations in eight out of the 14 kindreds with Bartter's syndrome. Most of these mutations were missense mutations, but two predicted truncations of the ROMK1 protein, consistent with their causing a loss of channel function.

These studies have shown that the primary abnormality in Bartter's syndrome lies within the epithelial cells of the medullary thick ascending limb (mTAL) of the nephron, and that mutations causing a loss of function to either the NKCC2 cotransporter or the ROMK1 channel may perturb the functioning of these cells sufficiently to produce impairment of sodium and chloride reabsorption. The consequent hypovolaemia activates renin and aldosterone secretion to produce an increase in distal tubular sodium reabsorption via the epithelial sodium channel (ENaC), which secondarily produces kaliuresis and compounds hypochloraemic alkalosis. Thus, the mechanism responsible for the hypokalaemic alkalosis in Bartter's syndrome is, at least in part, the same as that found in Liddle's syndrome, where increased ENaC function is due to a genetic activation of the channel, rather than due to the excess aldosterone stimulation found in Bartter's. About 20% of calcium reabsorption is also coupled to sodium reabsorption in the mTAL and therefore these defects also explain the hypercalciuria of Bartter's syndrome. As several other channels and transporters are also important in mTAL sodium and chloride reabsorption, it is possible that further genetic abnormalities may be found that cause Bartter's syndrome.

**Gitelman's syndrome**

Gitelman's syndrome (which is also referred to as Gitelman's variant of Bartter's syndrome) is also characterized by a hypokalaemic metabolic alkalosis, but in contrast to Bartter's syndrome, subjects have hypocalciuria and hypomagnesaemia as invariant features. Gitelman's syndrome occurs either as a sporadic or an autosomal recessive disorder and appears to be more prevalent in most populations than Bartter's syndrome. Subjects present in later childhood, adolescence or adult life with symptoms of hypokalaemia such as tetany, muscular weakness,
paraesthesiae or seizures.\textsuperscript{25,26} Although thirst and polyuria may occur, Gitelman’s subjects are not overtly hypovolaemic, but have activation of the renin-angiotensin II-aldosterone axis. Nephrocalcinosis is not a feature of Gitelman’s syndrome, as urinary calcium excretion is low; however, magnesium excretion is generally much higher than in Bartter’s syndrome, causing the marked hypomagnesaemia.\textsuperscript{26} Gitelman’s syndrome is usually associated with normal mental development. However, short stature is an occasional feature, and articular chondrocalcinosis can be a late complication.\textsuperscript{26,27}

The observation that the hypokalaemic, hypomagnesaemic metabolic alkalosis with hypercalciuria found in Gitelman’s syndrome resembled the biochemical picture caused by chronic thiazide diuretic use, prompted investigation of the distal convoluted tubular (DCT) thiazide-sensitive sodium-chloride cotransporter (NCCT) in Gitelman’s patients.\textsuperscript{28} NCCT is a 1021-amino-acid protein with 12 predicted transmembrane domains, and is similar in structure to NKCC2.\textsuperscript{25,28} The NCCT gene is located on the long arm of chromosome 16, and genetic linkage analysis revealed complete linkage of Gitelman’s syndrome to this chromosomal region in eight families.\textsuperscript{28} Mutational analysis of the NCCT in 12 Gitelman’s families showed 17 different mutations, which were either homozygous or compound heterozygous and spread throughout the length of the gene.\textsuperscript{28} Four of these 17 mutations are predicted to produce either a deletion or premature truncation of the NCCT protein and thus, since we know that thiazide diuretics (which are NCCT inhibitors) produce a similar effects to Gitelman’s, it seems likely that these mutations inactivate NCCT.

In contrast to the situation in Bartter’s syndrome, where there is a high incidence of consanguinity and therefore homozygous mutation of NKCC2, most Gitelman’s subjects are compound heterozygotes, and some families even have three mutant NCCT alleles, different combinations of which cause the phenotype in different generations.\textsuperscript{28} This suggests that heterozygotes with NCCT mutations may be common in the population, with a prevalence of up to 1%, and that there may be a selection advantage for such individuals.\textsuperscript{28} It has been suggested that this advantage may be a lower blood pressure,\textsuperscript{28} although it is difficult to envisage how this would improve fitness in those of reproductive age. Another possible explanation is that such heterozygotes are protected from nephrocalcinosis or tubular necrosis during periods of dehydration, by the hypocalciuria and defective urinary concentration, respectively. Furthermore, it is likely that subjects who carry one mutant NCCT allele are predisposed to the development of loop or thiazide-diuretic-induced hypokalaemic alkalosis.

### X-linked hypercalciuric nephrolithiasis

X-linked hypercalciuric nephrolithiasis, which is also known as Dent’s disease or X-linked recessive nephrolithiasis, is characterized by hypercalciuria, low molecular weight (LMW) proteinuria and progressive nephrocalcinosis, nephrolithiasis and renal failure.\textsuperscript{29–31} Other features include hypokalaemia, phosphaturia, renal glycosuria, amino aciduria and rickets, suggesting a generalized proximal tubulopathy of the Fanconi type.\textsuperscript{29–32} In males, the disorder usually presents in childhood with asymptomatic but substantial LMW proteinuria, associated with hypercalciuria or rickets. This progresses to nephrocalcinosis and nephrolithiasis in adolescence, and end-stage renal failure in early adult life.\textsuperscript{29–32} Renal transplantation is an effective therapy, with no recurrence of nephrocalcinosis in the graft, suggesting that the defect is intrinsic to the kidney. In females, a deterioration in renal function is extremely rare; LMW proteinuria and hypercalciuria are the only features observed.\textsuperscript{30,32}

The lack of male-to-male transmission in the nine families originally described with X-linked hypercalciuric nephrolithiasis, coupled with the milder phenotype in females, suggested that the abnormal gene product would be located on the X chromosome.\textsuperscript{30,31} Examination of genetic markers on the X chromosome revealed linkage to the proximal short arm (Xp11.22) in three families with this disorder, and affected males from one of these families also showed deletion of a single marker in this region.\textsuperscript{33,34} Fisher et al. isolated a candidate gene from the region of this deletion, which was expressed in renal tissue and was deleted in genomic DNA from affected males from this family.\textsuperscript{35} This gene product, CLC5, encoded a member of the family of voltage-gated chloride channels and was predicted to have 12 transmembrane domains.\textsuperscript{35} Subsequent mutational analysis of the CLC5 gene in a further ten kindreds with X-linked hypercalciuric nephrolithiasis showed point mutations or small deletions in all families.\textsuperscript{36} Many of these CLC5 mutations predicted deletions or premature truncations of the channel protein, and functional expression of the four missense CLC5 mutations in a Xenopus oocyte system confirmed a loss of CLC5 function.\textsuperscript{38} Further studies in a series of Japanese children with idiopathic LMW proteinuria, hypercalciuria and nephrocalcinosis\textsuperscript{37} have shown that this disorder is also caused by mutations in the CLC5 gene, and is therefore part of the spectrum of X-linked hypercalciuric nephrolithiasis.\textsuperscript{38} Clinical studies of females known to carry one abnormal CLC5 allele have now shown that LMW proteinuria is invariably present, and that hypercalciuria is present in about 50% of cases.\textsuperscript{32}
The mechanism by which loss of CLC5 function causes a generalized defect in tubular transport is unclear. Tissue distribution studies in the rat have shown that CLC5 mRNA is present along the entire length of the nephron, as well as in brain, lung and liver. One possible explanation is that either apical or basolateral CLC5 channels are responsible for maintaining the charge or volume status of the epithelial cell (or one of its intracellular) compartments throughout the nephron. The mutations found in X-linked hypercalciuric nephrolithiasis, which cause a loss of functional CLC5, may therefore result in electrically or osmotically unfavourable gradients across epithelial cell membranes and inhibition of reabsorption of each of the different substrates, e.g. LMW protein, glucose and phosphate in the proximal tubule, and sodium and calcium in the thick ascending limb.

**Summary**

Advances in the molecular genetics of inherited renal tubulopathies have allowed some insight into the normal mechanisms of tubular cation and anion reabsorption. It is now possible to view Bartter’s syndrome, Gitelman’s syndrome and pseudohypoaldosteronism type 1 as having genetic abnormalities which produce tubular defects that are similar to those induced by the pharmacological actions of loop diuretics, thiazide diuretics or potassium-sparing diuretics, respectively. Although these rare monogenic disorders with dramatic phenotypes seem to have little relevance to everyday clinical practice, it is possible that subtle abnormalities of the regulation of the ENaCs may play a role in low-renin forms of ‘essential’ hypertension. Similarly, subtle abnormalities in the function of the electroneutral sodium-potassium-chloride cotransporters (NKCC2 and NCCT) and the renal CLC-type chloride channels (CLC5) may be major determinants of urinary calcium excretion with roles in the pathogenesis of ‘idiopathic’ hypercalciuria and osteoporosis. Because of the intricate and diverse molecular mechanisms by which tubular reabsorption of water and solutes takes place in each different nephron segment, it is likely that other renal channels and transporters will be implicated in the pathogenesis of further monogenic disorders, and that these will allow additional insights into tubular functioning.

Recent studies have demonstrated that in addition to abnormalities in the NKCC2 and ROMK1 genes, mutations at a third genetic locus can also cause Bartter’s syndrome. Linkage studies, followed by mutational analyses have found deletions and point mutations in the gene encoding one of the TAL-specific chloride channels, CLCKB, in 17 Bartter’s families. This chloride channel is similar in structure to CLC5, and is located on the long arm of chromosome 17. Importantly, there appears to be a phenotypic difference between subjects with Bartter’s syndrome due to CLCKB abnormalities and those with NKCC2 or ROMK1 mutations. Despite the fact that all of these Bartter’s patients had significant hypercalciuria, nephrocalcinosis was not found in any of the 17 subjects with CLCKB mutations, compared to 19 of 20 patients with NKCC2 or ROMK1 mutations. These findings have also demonstrated a key role for CLCKB as a major basolateral chloride channel involved in mTAL sodium and chloride reabsorption (Figure 2).

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**References**

12. Kuhnle U, Nielsen MD, Tietze HU, Schoeber CH, Schlamp D,


