Salt-Losing Tubulopathies in Children: What’s New, What’s Controversial?

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ABSTRACT
Renal tubulopathies provide insights into the inner workings of the kidney, yet also pose therapeutic challenges. Because of the central nature of sodium in tubular transport physiology, disorders of sodium handling may affect virtually all aspects of the homeostatic functions of the kidney. Yet, owing to the rarity of these disorders, little clinical evidence regarding treatment exists. Consequently, treatment can vary widely between individual physicians and centers and is based mainly on understanding of renal physiology, reported clinical observations, and individual experiences. Salt-losing tubulopathies can affect all tubular segments, from the proximal tubule to the collecting duct. But the more frequently observed disorders are Bartter and Gitelman syndrome, which affect salt transport in the thick ascending limb of Henle’s loop and/or the distal convoluted tubule, and these disorders generate the greatest controversies regarding management. Here, we review clinical and molecular aspects of salt-losing tubulopathies and discuss novel insights provided mainly by genetic investigations and retrospective clinical reviews. Additionally, we discuss controversial topics in the management of these disorders to highlight areas of importance for future clinical trials. International collaboration will be required to perform clinical studies to inform the treatment of these rare disorders.

The preservation of electrolyte, volume, and acid-base homeostasis balance is vital to the functioning of our bodies. Because life initially evolved in the ocean, cellular function is dependent on the maintenance of the electrolyte concentration reflective of the original environment. No idea could be thought, no muscle moved, without the proper balance of salts within our bodies. It is the responsibility of mainly the kidneys to maintain this vital “milieu intérieur.” The kidneys do so by the combination of glomerular filtration and tubular reabsorption, a system that is best explained by the evolutionary history. “Intelligent design” may not have devised a system that, in an average adult, initially filters approximately 150 L of water daily, containing an enormous load of solutes, including about 20,000 mmol of sodium per day (equivalent to the amount in 1.2 kg of cooking salt), only to laboriously reabsorb virtually all of it back into circulation. In the original ocean environment, a system of filtration was well suited given the unlimited availability of salt and water. Yet, in order to enable life on land, large losses of water and solutes had to be prevented, leading to the evolution of ever more powerful tubules. Under physiologic conditions, they are capable of reabsorbing >99% of filtered sodium and water. This enormous task is accomplished by a combination of distinct sodium or sodium-coupled transport systems along the nephron. It is the active reabsorption of sodium that generates the main driving force for the passive reabsorption of water. The price to pay for this powerful system of filtration and reabsorption is a high-energy demand: when adjusted for organ weight, the kidneys, together with the heart, have the highest resting metabolic expenditure, approximately 440 kcal/kg per day, almost twice as much as the brain, making the kidneys susceptible to acute injury when the energy supply is impaired.

The importance of tubular sodium reabsorption becomes especially apparent when the system is disturbed, as in the salt-losing tubulopathies. On the basis of anatomic and functional characteristics, the tubules are typically divided into four main segments: proximal tubule (PT), thick ascending limb (TAL) of Henle’s loop, distal convoluted tubule (DCT), and collecting duct (CD). Genetic or acquired defects in salt transport in any of these segments lead to distinct tubulopathies, which have characteristic clinical and biochemical features (Figure 1).

Interestingly, given the critical nature of sodium for the maintenance of volume homeostasis, essentially all salt-losing tubulopathies actually maintain normal
sodium excretion in steady state, because persistent losses exceeding intake would be incompatible with life. This normal sodium excretion is achieved by a compensatory increase in absorption through other pathways. This explains the commonly seen hyperaldosteronism that characterizes, for instance, Bartter and Gitelman syndromes (BS and GS, respectively), and that enhances sodium reabsorption in the CD, primarily at the expense of potassium secretion. Consequently, a key diagnostic feature for renal salt wasting is the fractional excretion of chloride.4

Here, we will review key advances in our understanding of the different renal genetic disorders affecting salt (sodium chloride) reabsorption with respect to both clinical phenotype and underlying pathophysiology. An overview of these disorders, the underlying genes, and key clinical characteristics is given in Table 1. In addition, we will highlight some clinical controversies around the treatment of salt-losing tubulopathies, which need further clinical studies and which are summarized in Table 2.

**PT**

The PT is the part of the nephron where the most diverse action with respect to reabsorption (as well as secretion) takes place (Figure 2). Micropuncture studies suggest that between 60% and 80% of all filtered salt and water is reabsorbed in this segment.5 The “engine” for salt transport, as in all tubular segments, is the basolateral Na⁺-K⁺-ATPase, which establishes the electrochemical gradient for sodium entry into the cell. The Fanconi renotubular syndrome (FRTS) represents global dysfunction of the PT and, because of the high energy demand of this transport process, is often associated with disorders of impaired energy supply, such as mitochondrial cytopathies.6 But, aside from being a secondary feature of systemic disorders, FRTS can also occur in primary form. Currently, OMIM lists three such forms of FRTS (see Table 1): FRTS1 is dominantly inherited and typically presents in childhood with rickets and the typical biochemical abnormalities of FRTS. Progressive CKD is typically observed with development of CKD stage 5 in adulthood. Although the underlying gene has been linked to a locus on chromosome 15, the identity of this gene remains to be revealed.7 FRTS2 previously referred to FRTS observed in two siblings with a homozygous mutation in SLC34A1.8 Yet, recessive mutations in this gene were subsequently found to cause infantile hypercalciuria and the association with FRTS has been questioned.9 A unique aspect of PT energy utilization was highlighted by our discovery of the molecular basis of FRTS type 3, where a het erozygous missense mutation in EH-HADH (encoding enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase) impairs mitochondrial fatty acid oxidation.10,11 Although this defect is global it only manifests in the PT, because the PT does not utilize glucose for energy generation, exposing the dependency on fatty acid oxidation.12 Patients typically present in childhood with rickets and the biochemical abnormalities. In contrast to FRTS1, however, no progressive CKD has been observed.13 FRTS4 is caused by a specific mutation (R76W, also annotated as R63W, depending on reference sequence) in the transcription factor HNF4A.14 Mutations in this gene are associated with abnormalities in insulin secretion, typically hyperinsulinemic hypoglycemia manifesting in the neonatal period and diabetes (MODY type 1) later in life. Consequently, patients with FRTS4 usually manifest shortly after birth with hypoglycemia and subsequent investigations then reveal the FRTS.15,16 The association of FRTS4 with only this one specific mutation (all other described HNF4A mutations are only associated with altered insulin secretion) raises interesting questions over the specific role of R76 for the function of HNF4A in the maintenance of proximal tubular function, but, so far, no insights have been published.

The bulk of sodium in the PT is taken up by the apical Na⁺-H⁺ exchanger NHE3, in this way linking sodium and thus volume homeostasis with acid-base homeostasis.17 Given this important role, one might have expected a severe form of a salt-losing tubulopathy and proximal acidosis with loss of function of this transporter. Instead, recessive mutations in the encoding gene SLC9A3 are associated with congenital sodium diarrhea (OMIM #616868).18 Only two of the seven reported patients with available data exhibited acidosis. While also presenting with diarrhea, mice lacking Nhe3 function do also show evidence of salt wasting and acidosis.19 To better dissect the respective renal and/or intestinal contribution to the acidosis, a renal

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**Figure 1.** Sodium and water reabsorption along the nephron. Knockouts of distinctive proteins in particular nephron segments lead to distinctive disease in man, as indicated.
<table>
<thead>
<tr>
<th>Nephron Segment</th>
<th>Disorder</th>
<th>OMIM</th>
<th>Gene</th>
<th>Protein</th>
<th>Inheritance</th>
<th>Typical Clinical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>Fanconi renotubular syndrome type 1; FRTS1 Infantine hypercalciuria 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134600</td>
<td>SLC34A1</td>
<td>NaPi-IIa</td>
<td>AR/AD</td>
<td>Rickets, progressive CKD, hypercalciuria, nephrocalcinosis</td>
</tr>
<tr>
<td></td>
<td>Hypophosphatemic rickets with hypercalciuria</td>
<td>613388</td>
<td>SLC34A3</td>
<td>NaPi-Iic</td>
<td>AR/AD</td>
<td>Hypercalciuria, nephrocalcinosis</td>
</tr>
<tr>
<td></td>
<td>Fanconi renotubular syndrome type 3; FRTS3</td>
<td>241530</td>
<td>EHHADH</td>
<td>EHHADH</td>
<td>AD</td>
<td>Rickets, no kidney failure</td>
</tr>
<tr>
<td></td>
<td>Fanconi renotubular syndrome type 4; FRTS4</td>
<td>615605</td>
<td>HNF4A</td>
<td>HNF4A</td>
<td>AD</td>
<td>Congenital hyperinsulinism, later MODY, rickets</td>
</tr>
<tr>
<td></td>
<td>Renal glucosuria</td>
<td>233100</td>
<td>SLC5A2</td>
<td>SGLT2</td>
<td>AR/AD</td>
<td>Glucosuria</td>
</tr>
<tr>
<td></td>
<td>Proximal renal tubular acidosis with eye findings</td>
<td>604278</td>
<td>SLC4A4</td>
<td>KNBC</td>
<td>AR</td>
<td>Metabolic acidosis, eye abnormalities (corneal opacities, band keratopathy, cataract, glaucoma), mental impairment</td>
</tr>
<tr>
<td></td>
<td>Osteopetrosis with renal tubular acidosis</td>
<td>259730</td>
<td>CA2</td>
<td>CA2</td>
<td>AR</td>
<td>Osteopetrosis, cerebral calcifications, metabolic acidosis</td>
</tr>
<tr>
<td>TAL</td>
<td>BS type 1</td>
<td>601678</td>
<td>SLC12A1</td>
<td>NKCC2</td>
<td>AR</td>
<td>Prematurity, polyhydramnios, nephrocalcinosis, hypokalemic alkalosis, iso- or hyposthenuria</td>
</tr>
<tr>
<td></td>
<td>BS type 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>241200</td>
<td>KCNJ1</td>
<td>ROMK1</td>
<td>AR</td>
<td>Prematurity, polyhydramnios, nephrocalcinosis, transient hyperkalemia, then hypokalemic alkalosis, iso- or hyposthenuria</td>
</tr>
<tr>
<td></td>
<td>BS type 3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>607364</td>
<td>CLCNKB</td>
<td>CIC-Kb</td>
<td>AR</td>
<td>Severe hypokalemic hypochloremic alkalosis</td>
</tr>
<tr>
<td></td>
<td>BS type 4A&lt;sup&gt;h&lt;/sup&gt;</td>
<td>602522</td>
<td>BSND</td>
<td>Barttin</td>
<td>AR</td>
<td>Prematurity, polyhydramnios, sensorineural deafness, severe hypokalemic hypochloremic alkalosis, iso- or hyposthenuria</td>
</tr>
<tr>
<td></td>
<td>BS type 4B&lt;sup&gt;h&lt;/sup&gt;</td>
<td>607364</td>
<td>CLCNKA, CLCNKB</td>
<td>CIC-Ka, CIC-Kb</td>
<td>AR</td>
<td>Severe hypokalemic hypochloremic alkalosis, iso- or hyposthenuria</td>
</tr>
<tr>
<td></td>
<td>BS type 5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>300971</td>
<td>MAGED2</td>
<td>MAGED2</td>
<td>XR</td>
<td>Severe polyhydramnios, transient salt wasting</td>
</tr>
<tr>
<td></td>
<td>AD hypocalcemic hypercalciuria</td>
<td>263800</td>
<td>SLC12A3</td>
<td>NCC</td>
<td>AR</td>
<td>Hypocalcemic hypercalciuria, that can be complicated by hypokalemic alkalosis</td>
</tr>
<tr>
<td>DCT</td>
<td>EAST syndrome</td>
<td>612780</td>
<td>KCNJ10</td>
<td>Kir4.1</td>
<td>AR</td>
<td>Epilepsy, ataxia, sensorineural deafness, hypokalemic alkalosis</td>
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</tbody>
</table>
specific knock-out was generated, which confirmed renal bicarbonate wasting, albeit with only mild acidosis. These studies confirm the important role of NHE3; yet, at least in PT, the loss of function may be partially compensated by other NHE isoforms, such as NHE8.

Another important sodium transporter in PT is the Na⁺-PO₄⁻ cotransporter NaPi-IIa, encoded by SLC34A1. Initially, a homozygous loss-of-function mutation was reported as the cause of FRTS type 2 in two siblings. Yet, no further patients with FRTS and SLC34A1 mutations have been identified since. Instead, recessive loss-of-function mutations in this gene are recurrently found as the cause of infantile hypercalciuria with nephrolithiasis (OMIM #612286). Moreover, heterozygous mutations have been associated with hypophosphatemic rickets with hypercalciuria caused by heterozygous mutations in SLC34A1, encoding NaPi-IIc (OMIM #241530). Presumably, the hypophosphatemia-mediated suppression of FGF23 leads to increased 1-α hydroxylation of cholecalciferol with resultant hypercalciemia and hypercalciuria.

Of interest is also the sodium-glucose cotransporter SGLT2, encoded by SLC5A2. Recessive mutations in this transporter cause isolated renal glucosuria (OMIM #233100), which provides an example of the enormous benefits the study of a rare disorder can have for common disorders. Patients with isolated renal glucosuria can lose well over 100 g of glucose daily, yet with no apparent detrimental consequences. In fact, in an era of affluence in which obesity and diabetes have become major threats to public health, the loss of sodium and glucose in the urine may be beneficial and SGLT2 thus became an attractive therapeutic target. Inhibitors of SGLT2, the gliflozins, are now available and seem to provide substantial benefits in the management of diabetes, with not only improved glucose control but also reduced cardiovascular mortality and diabetic nephropathy.

Two further transporters affecting sodium reabsorption in the PT are associated with diseases: recessive mutations in SLC4A4, encoding basolateral Na-bicarbonate cotransporter NBC1, cause proximal tubular acidosis with eye findings (OMIM #604278); and recessive mutations in carbonic anhydrase 2, encoded by CA2, cause proximal tubular acidosis with osteopetrosis (OMIM #259730). Although CA2 does not

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Table 1. Clinical controversies discussed in this review. These controversies need clarification through clinical trials and/or expert consensus. ACEi, angiotensin-converting enzyme inhibitors; COXi, cyclooxygenase inhibitors.

<table>
<thead>
<tr>
<th>Tubular Segment</th>
<th>Clinical Controversies</th>
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</thead>
<tbody>
<tr>
<td>PT</td>
<td>Do antiproteinuric drugs such as ACEi protect kidney function, if proteinuria is of tubular origin?</td>
</tr>
<tr>
<td>TAL</td>
<td>Is salt supplementation beneficial in patients with secondary NDI?</td>
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<tr>
<td></td>
<td>Which COXi provides best efficacy with least side effects: indomethacin, ibuprofen, or celecoxib?</td>
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<tr>
<td></td>
<td>Should COXi be weaned off during school age or should they be maintained life-long?</td>
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<tr>
<td></td>
<td>Is antenatal treatment of BS beneficial?</td>
</tr>
<tr>
<td>TAL and DCT</td>
<td>What is the best classification for BS and GS?</td>
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<td></td>
<td>What is the lower limit of plasma potassium concentration that can be considered safe?</td>
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<tr>
<td></td>
<td>Are potassium-sparing diuretics beneficial?</td>
</tr>
<tr>
<td></td>
<td>Are antiproteinuric drugs indicated in patients who have developed proteinuria?</td>
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<tr>
<td></td>
<td>What is the lower limit of plasma magnesium concentration that can be considered safe?</td>
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<tr>
<td></td>
<td>Is salt supplementation alone sufficient to normalize renin and aldosterone levels?</td>
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<tr>
<td>DCT</td>
<td></td>
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</tbody>
</table>

Listed are clinical controversies discussed in this review. These controversies need clarification through clinical trials and/or expert consensus. ACEi, angiotensin-converting enzyme inhibitors; COXi, cyclooxygenase inhibitors.
transport sodium directly, it clearly affects the availability of H\(^+\) for the apical Na\(^+\)-H\(^+\) exchange. However, except for occasional case reports and case series, very little new data are available for these very rare disorders.\(^28,29\)

**Clinical Controversies**

Patients with FRTS exhibit proteinuria. This primarily reflects “tubular” proteinuria, i.e., the impaired reabsorption of filtered proteins, but in some patients proteinuria reaches the nephrotic range.

Although the exact amount of physiologic tubular protein (including albumin) reabsorption remains controversial and may approach several grams per day,\(^30\) some patients also show evidence of glomerular damage.\(^31,32\) Should these patients be treated with so-called antiproteinuric drugs, such as ACEi or ARB? Given the impaired sodium reabsorption in FRTS and thus risk of loss of volume homeostasis, are such treatments, which are generally recommended for proteinuria in CKD, of benefit to patients with rare tubular salt-losing disorders?

**THICK ASCENDING LIMB**

From the PT, the primary urine traverses into the thin limb of the loop of Henle. So far, no human disorders have been associated with altered salt transport here, so we will concentrate on the thick ascending limb (TAL, Figure 3).

The study of rare disease has greatly enhanced our understanding of this nephron segment, mainly through investigations into BS and familial hypomagnesemia with hypercalciumia and nephrocalcinosis (FHHNC). The electroneutral furosemide-sensitive Na\(^+\)-2Cl\(^-\)-K\(^+\) cotransporter (NKCC2), encoded by SLC12A1, is the key apical sodium transporter. Mutations in this gene cause BS type 1 (OMIM #601678). Function of NKCC2 is dependent on apical recycling of potassium through the potassium channel ROMK, encoded by KCNJ1. Consequently, mutations in this channel are the cause of BS type 2 (OMIM #241200). Together, these two transport proteins generate the lumen positive transepithelial potential, which drives the paracellular absorption of calcium and magnesium. Sodium exits the cell on the basolateral (blood side) via the Na-K-ATPase, whereas chloride exits through the chloride channels CLCNKB (defective in Bartter type 3) and CLCNKA; both require Barttin (defective in Bartter type 4) for proper membrane localization. NKCC2 can be inhibited by loop diuretics, such as furosemide.

![Figure 2. Simplified diagram of a PT cell. Sodium reabsorption in the PT is mainly accomplished by NHE3, which exchanges sodium for protons. Other sodium-coupled transporters use the chemical and electrical gradient of sodium for the reabsorption of molecules (X stands for, e.g., glucose, amino acids, phosphate).](image)

![Figure 3. Simplified diagram of a TAL cell. Sodium is reabsorbed electroneutrally via NKCC2 (defective in Bartter type 1), together with one potassium and two chloride ions. The transporter can only function with all four ions bound and, because of its luminal concentration, potassium binding becomes the rate-limiting step. Therefore, potassium is recycled through the potassium channel ROMK1 (defective in Bartter type 2) to ensure an adequate luminal supply of potassium. This also generates a lumen positive transepithelial potential, providing the driving force for paracellular absorption of calcium and magnesium. Sodium exits the cell on the basolateral (blood side) via the Na-K-ATPase, whereas chloride exits through the chloride channels CLCNKB (defective in Bartter type 3) and CLCNKA; both require Barttin (defective in Bartter type 4) for proper membrane localization. NKCC2 can be inhibited by loop diuretics, such as furosemide.](image)
of sodium and chloride is mediated by the Na\(^+\)-K\(^+\)-ATPase and the chloride channel CLCNKB, respectively. Recessive mutations in CLCNKB are the cause of BS type 3 (OMIM #607364). It is likely that the close homolog CLCNKA contributes to salt reabsorption in TAL, explaining the typically more severe phenotype in patients lacking Barttin (BSND), the obligate subunit for both CLCNK homologs, mutations in which cause BS type 4A (OMIM #602522). A similar severe phenotype occurs with loss-of-function mutations in both chloride channels (BS type 4B, OMIM #613090). In contrast to these observations in patients, data from mouse studies do not support the notion of a substantial contribution of ClC-K1 (the mouse ortholog of CLCNKA) to salt reabsorption in TAL.\(^{34,35}\) It is important to note that CLCNKB and Barttin constitute the key pathway for basolateral chloride exit also in the DCT (see below) and thus can phenotypically resemble a mixed TAL/DCT disorder.\(^{34,36}\)

Previously, a terminology had been proposed to separate BSs into so-called antenatal BS (BS types 1, 2, and 4) and “classic” BS (BS type 3) with presentation later in childhood.\(^{37}\) Indeed, retrospective reviews clearly show a trend for more severe antenatal presentation in BS types 1, 2, and 4 compared with type 3.\(^{3,48}\) Yet, there is a wide spectrum of severity in all forms of BS: some patients with BS type 1, 2, or 4 present only later in life, including adulthood, whereas some patients with BS type 3 have a severe antenatal presentation with prematurity as early as 22 weeks of gestation and polyhydramnios treated with amniocentesis.\(^{39-41}\) There are some data for CLCNKB suggesting that mutation type may influence the phenotype, with mutations affecting the Barttin-binding site, the dimerization interface, or the selectivity filter causing more severe dysfunction.\(^{42}\) Yet, the most common mutation in CLCNKB is a whole-gene deletion, which can be associated with the whole phenotypic spectrum. One recent review of 30 patients with BS type 3 found no evidence for a genotype-phenotype association,\(^{43}\) whereas in a larger series of 115 patients an association of complete loss-of-function mutations with age at onset was seen.\(^{46}\)

### Regulation by MAGED2

Previously, transient forms of antenatal BS have been described, but it was only recently that a genetic explanation for a subset of these patients was identified: loss-of-function mutations in the melanoma-associated antigen-D2 (MAGED2).\(^{47}\) It appears that the encoded protein is an important regulator of tubular salt reabsorption in TAL and DCT in the ante- and perinatal period, but not thereafter. The gene maps to the X-chromosome and pregnancies with affected boys are prominently characterized by severe polyhydramnios.\(^{47}\) After delivery, polyuria persists with hypokalemic alkalosis, but symptoms resolve spontaneously during the first few months of life. Why there seems to be separate regulation of tubular transport before and after birth, and what role exactly MAGED2 plays in this process, remain to be elucidated.

### Macula Densa, Tubuloglomerular Feedback, and Hyperreninism

Hypertrophy of the juxtaglomerular apparatus (JGA) was already part of the original description of BS.\(^{48}\) The JGA is at the interface of glomerular and tubular function and mediates tubuloglomerular feedback (TGF) and essentially constitutes the “volume sensor” of the kidney, where on the basis of tubular sensing, renin release and glomerular filtration are regulated.\(^{49}\) The tubular component of the JGA is the macula densa and chloride transport is a key initial signaling pathway: decreased chloride availability indicates inadequate filtration and leads to activation of the TGF with consequent renin release andafferent arteriolar dilatation with hyperfiltration through a number of intermediate steps, most prominently the production of PGE\(_2\) (PGE2) by cyclooxygenase-2 (COX2).\(^{50,51}\) This elevated COX2 activity is the basis for treatment of BS with PG synthesis inhibitors. Importantly, because macula densa cells are part of the TAL, mutational effects are present here as well, leading in essence to a short-circuit of TGF, as chloride reabsorption is genetically impaired in BS. Consequently, renin release and regulation of filtration are essentially uncoupled from volume status in BS, because the volume sensor is defective. PGE2 production appears be highest in BS types 1 and 2, which led to the proposal of the term “hyperprostaglandin E syndrome” for these BS subforms.\(^{37}\) Yet, despite the lower PG levels, the hypokalemic alkalosis is typically much more pronounced in BS type 3 compared with types 1 and 2.\(^{38,52}\) The reasons for this are not quite clear: it is, because CLCNKB is expressed also beyond TAL in the DCT, thus impairing salt transport in two nephron segments.\(^{35}\) But why then is it typically the “milder” form with later onset? Do patients with BS type 3 have higher aldosterone levels, despite lower PGE2? If so, what triggers the aldosterone production? Further investigations are needed to better understand the often dramatic electrolyte abnormalities in BS3.

### Clinical Controversies

The wide spectrum of clinical severity in all types of BS has led to controversies regarding the terminology: should a classification system be on the basis of the clinical phenotype, distinguishing between “antenatal” BS (sometimes also referred to as “hyperprostaglandin E syndrome”) and “classic” BS?\(^{33}\) But where exactly is the separation? Does a patient born at 36 weeks have antenatal or classic BS? Or should it be on the basis of the clinical similarity to the effects of diuretics and thus the predominantly affected nephron segment: loop versus DCT disorder?\(^{37}\) But where do patients belong, who initially have a BS-like phenotype but later fit a GS type, as can be seen in patients with CLCNKB mutations?\(^{46}\) Do they switch classification and thus are told at some point that they have a different diagnosis then initially assigned? Or should we stick with the genetic classification, as in this review? But even there is heterogeneity: BS type 5 is referred to by some authors as related to mutations in CASR, by others to combined mutations in CLCNKA and CLCNKB, and more recently it has
been assigned by OMIM to the transient BS associated with mutations in MAGED2 (see Table 1).47-54 It gets even more confusing when clinical and genetic criteria are combined, so that antenatal BS becomes synonymous with BS types 1, 2, and 4, and classic BS with type 3.55 In this system, a patient with adult presentation and mutations in SLC12A1 would be categorized as antenatal BS, whereas the premature baby with CICNKB mutations would have classic BS. Similarly, a baby with BS born prematurely after a pregnancy complicated by polyhydramnios could be classified either as antenatal or classic BS, depending on the underlying genetic cause. Although such a classification system captures well the majority of patients, in a substantial minority (including the 20%-30% of patients with BS type 3 with antenatal presentation) the clinical and genetic criteria diverge.58,46

Potentially severe complications related to the electrolyte abnormalities, such as cardiac arrhythmias, have been described in BS.55 We recently described a case of BS type 4 with such dramatic alkalosis that not only was there impaired breathing (“renal apnea”), but there also seemed to be a more generalized enzyme dysfunction.56 Alkalosis is typically most severe in patients with BS types 3 and 4, potentially related to chloride depletion, emphasizing the need to use chloride-containing salt supplementation.57 Indeed, stabilization of volume status with salt and water should always be the first therapeutic aim. Yet, even the combined use of such supplementation with PG synthesis inhibitors is often not sufficient to achieve sustained normalization of electrolyte abnormalities. This is most dramatically seen in BS type 4.58 So, what level of potassium can we consider safe and should we aim for? Is a wildly swinging potassium level associated with large doses of intermittent supplementation safer than a stable but very low level? The hypokalemic alkalosis can be improved with the use of K+-sparing diuretics, but this is controversial: BS is primarily a salt-wasting disorder and the salt wasting will be compounded by the use of K+-sparing diuretics, putting the patient at risk of severe hypokalemia. Although we can measure potassium very easily and thus may feel prompted to treat abnormalities, hypokalemia is much more difficult to express in exact numbers. Could some of the reported sudden collapses in salt-losing tubulopathies be related to hypovolemia rather than hypokalemia; for instance, when the patient develops vomiting and/or diarrhea, compounding the renal with intestinal salt losses? However, given the above discussed short-circuit of the JGA in BS, where renin production appears to be uncoupled from volume status, the use of K+-sparing diuretics may be justified, as long as volume is sufficiently supported by fluid and pharmacologic salt supplementation. Further studies to assess the efficacy of these drugs are needed. If used, amiloride may be preferable to mineralocorticoid antagonists, such as spironolactone, because the alkalosis may not only be mediated by H+ secretion in the aldosterone-sensitive distal nephron alone, but also by NHE3 in TAL, which is also inhibited by amiloride, albeit with a much lower affinity compared with ENaC.59

Obviously, this controversy extends beyond potassium-sparing diuretics, but also concerns the use of angiotensin converting enzyme inhibitors and angiotensin receptor blockers. Although not commonly used in BS, physicians may be inclined to use them in those patients who develop proteinuria, especially if the biopsy reveals glomerulosclerosis.46 Whether these medications at this stage can help protect kidney function that could justify the risk of hypotension is yet another open question.

Another treatment dilemma occurs in patients with BS complicated by a nephrogenic diabetes insipidus (NDI)-like phenotype. The TAL is critical for urinary dilution and the establishment of the interstitial concentration gradient.60 Consequently, isoosmhenuria, the inability to either dilute or concentrate the urine, would be expected in BS and is indeed present in many of them. However, there are some patients with NDI-like features, i.e., with a urine osmolality <200 mosm/kg and an inability to concentrate the urine after administration of DDAVP,61,62 Misdiagnosis of such patients with BS as primary NDI has been reported and we therefore suggested the term “secondary inherited NDI.”63 Presumably, the urinary dilution is mediated by a hypertrophied DCT, but the unresponsiveness to DDAVP remains to be clarified. Clinically, these patients present a treatment dilemma: salt supplementation is usually a key component of the treatment of BS, but contraindicated in NDI. In our own clinical experience, salt supplements are poorly tolerated by these patients, and often associated with hypernatremia and increased thirst (unpublished observations).

Since the discovery of elevated renal production of PGs and their role in mediating elevated renin and aldosterone levels, suppression of PGE2 production by PG synthesis inhibitors is generally accepted as beneficial in the treatment of BS, at least during the first few years of life.64,65 Yet, during later childhood, this effect appears to be less pronounced and PG synthesis inhibitors are often weaned or withdrawn. Why is this? Is it because patients can now self-regulate their salt and water intake and thus better self-maintain homeostasis? Or are there developmental changes in the regulation of tubular salt reabsorption that make PGs less relevant? The discovery of BS type 5 and the causative gene MAGED2 clearly provide evidence for developmental changes in the first months of life in the regulation of tubular salt transport.66

In the infantile period, clinical observations demonstrate an often dramatic improvement from PGE2 inhibition: it reduces polyuria, improves the electrolyte abnormalities, and ameliorates the failure to thrive.64 However, which drug should be used? The most commonly prescribed drug in BS is indomethacin due to its described efficacy with respect to decreased polyuria, improved growth, and electrolyte control.67,68 Yet, it can also be associated with severe side effects, especially
gastrointestinal, such as gastric ulcer, necrotizing enterocolitis, and gut perforation. Moreover, long-term use as pain medication is associated with CKD. The identification of the critical role of COX2 in the excess production of PGs in BS established selective COX2 inhibitors, the so-called “coxibs,” as a promising new treatment option. Indeed, successful treatment with these drugs has been recurrently reported. However, with the realization of the increased cardiovascular mortality with selective compared with nonselective COX inhibitors, at least in adults, and the subsequent withdrawal of rofecoxib from the market, the use of the coxibs in BS has remained controversial. In one retrospective review of 28 patients with BS or GS, rofecoxib use was associated with higher BP compared with indomethacin, although in both groups BP was still below the average. It remains to be determined what poses the greater threat to patients with BS: the risk of gastrointestinal and/or renal side effects, or the potentially increased cardiovascular mortality. Yet, even with regard to the renal side effects, there is some controversy: retrospective reviews have revealed that a substantial subset of patients (up to 25%) with BS develop CKD. The reasons for this may include prematurity and indomethacin nephrotoxicity. Yet, interestingly, if biopsies are performed, these typically do not reveal the tubulointerstitial changes expected from indomethacin toxicity, but often glomerulosclerosis. Consequently, it has been speculated that the persistent elevation of PGs with consequent glomerular hyperfiltration and elevated renin and aldosterone may contribute to this glomerular damage, and that life-long treatment with PG synthesis inhibitors may therefore actually protect, rather than impair, long-term kidney function. Lastly, the antenatal treatment of BS has been reported in isolated cases. Yet, whether there is true benefit from this early treatment which justifies the increased risk of potential complications such as necrotizing enterocolitis remains controversial.

DCT

Impaired salt reabsorption in the DCT is associated with two disorders: GS (OMIM #263800) and EAST syndrome (also called SeSAME, OMIM #612780). GS is probably the most common renal salt-wasting disorder with an incidence of around 1:25,000 and is often considered a mild disorder. It is typically diagnosed during adolescence or adulthood, often incidentally, when blood tests are obtained for other reasons and hypokalemia is noted. Yet, there are many patients who report significant symptoms, such as severe fatigue, lack of stamina, and impaired quality of life. Interestingly, the severity of electrolyte abnormalities in these patients is not significantly different from those with minor or no symptoms. Later in life, patients may develop complications, such as chondrocalcinosis and sclerochoroidal calcifications. Surprisingly, the development of hypertension has been reported in isolated adult patients with GS and one large retrospective study suggests that there may also be an increased risk of type 2 diabetes and CKD.

From an isolated renal perspective, EAST syndrome is essentially indistinguishable from GS and the severity of the disorder is primarily determined by the extrarenal manifestations: generalized seizures are commonly the presenting syndrome in infancy, yet the epilepsy typically improves with time and many patients have several years with little or no seizure activity, although with later emergence of focal epilepsy. Although KCNJ10 is expressed in the eye and patients have subtle, but distinct, changes on electoretinograms, these do not lead to any apparent symptoms. It is the degree of ataxia, especially with the associated speech dyspraxia, that determines mostly the disability of patients, with some patients virtually unable to communicate because both speech and written communication are impaired by the movement disorder. This decreased expressive ability may have contributed to the stigmatizing label of “mental retardation,” part of the acronym “SeSAME.”

The identification of animal models of EAST syndrome may facilitate the generation of new treatments for this severe multisystem disorder.

The discovery of EAST syndrome established the critical role of KCNJ10 for the function of the DCT and prompted the consideration for the DCT as the renal “K+-sensor” to maintain potassium homeostasis. Hypokalemia leads to hyperpolarization of the basolateral membrane of DCT cells with consequent enhanced chloride exit through CLCNKB. The resultant decreased intracellular chloride concentration activates the WNK-SPAK pathway, which in turns leads to phosphorylation and thus increased activity of the NaCl cotransporter NCC, so that little sodium remains to be delivered to the CD. Conversely, in hyperkalemia, sodium uptake in DCT will be decreased and more sodium is delivered to the CD where sodium reabsorption can be balanced by potassium secretion (see Figures 4 and 5). The WNK kinases WNK4 and WNK1 are important regulators for the relative abundance of sodium reabsorption in DCT versus CD, and the interplay between sodium and potassium is increasingly recognized as a key element not only for potassium homeostasis, but also BP regulation.

The expression of CLCNKB in DCT also explains why patients with BS type 3 can phenotypically resemble GS. Patients with HNF1B mutations can also phenocopy GS, presenting the typical electrolyte profile, sometimes with only minor radiologic abnormalities of the kidneys, leading to a potential misdiagnosis.

Clinical Controversies

The same potentially serious complications of hypokalemic alkalosis reported in BS have also been associated with GS. However, because GS does not involve the JGA and thus TGF is intact, potential elevations of PGs, renin, and aldosterone should reflect physiologic compensation for the salt losses in the DCT. Following this understanding of the pathophysiology, such activation of the renin-angiotensin system should be suppressible
by sufficient salt supplementation and, consequently, treatment with PG synthesis inhibitors and/or K⁺-sparking diuretics should be avoided in GS. Nevertheless, a beneficial effect of such drugs in GS has been reported.⁹⁷ Apparently, despite our insights into renal pathophysiology, we still do not fully understand the development of symptoms in GS. Yet, in our experience, seizures occur in EAST syndrome independent of plasma magnesium levels and, similarly, symptoms of GS cannot be correlated with the degree of electrolyte abnormalities.⁸³,⁸⁵

An increase in plasma levels after oral supplementation results in increased glomerular filtration and thus increased renal losses, leading to a virtual impossibility in many patients to normalize plasma levels with oral supplementation.

**Figure 5.** Simplified diagram of a principal cell and type 1 intercalated cell in the cortical CD. Sodium reabsorption occurs electronegatively through ENaC and Na-K-ATPase and thus facilitates potassium and proton secretion through ROMK and the H⁺-ATPase, respectively. Aldosterone indirectly affects the activity of these proteins via the mineralocorticoid receptor MRCR. ENaC can be inhibited by amiloride.

**CD**

The CD is the final part of the nephron and, although quantitatively the smallest proportion of filtered sodium is reabsorbed here, this is the tubular segment where final decisions about sodium and water reabsorption are being made and reabsorption is most regulated. Because there is no further segment downstream to compensate, dysfunction in this segment can be most devastating, as seen in the autosomal recessive form of pseudohypoaldosteronism type 1 (arPHA1, OMIM #264350), caused by loss-of-function mutations in any of the subunits of the epithelial sodium channel ENaC. Affected infants typically present in the first few days with excessive weight loss and are found to have life-threatening hypovolemia with hyperkalemia and acidosis. Interestingly, PHA1 is the only salt-losing tubulopathy that typically presents with hyponatremia, presumably because the severe hypovolemia leads to release of antidiuretic hormone. Because ENaC is also expressed in lungs and skin, patients with PHA1 can also suffer from cystic fibrosis–like lung disease, as well as from a miliary skin rash. In contrast, the autosomal dominant form (adPHA1, OMIM #177735) has exclusive renal manifestations. It is caused by heterozygous mutations in NR3C2, the gene encoding the mineralocorticoid receptor. Affected patients typically present in the first month of life with insufficient weight gain and subsequent blood tests show hyponatremia, hyperkalemia, and
Much has been learned about salt-losing tubulopathies, catalyzed by the recent advances in genetics, which has led to the identification of most of the underlying genes. However, clinical observations in genotyped patients continue to raise questions about specific aspects of the roles these genes. Most importantly, despite the ever more detailed insights into human physiology, treatment for most these disorders is highly variable between physicians and sometimes even controversial. Fundamental questions, such as whether hypokalemia or hypovolemia pose a graver threat to patients with BS and GS, remain open. Because of the rarity of these disorders, solid clinical evidence is usually not available and it is the anecdotal experience that often influences the individual physician. National and international efforts, such as the United Kingdom registry for rare renal diseases (www.rarerenal.org), the Kidney Disease Improving Global Outcomes expert consensus conference on GS, and the European Reference Network for rare diseases (http://ec.europa.eu/health/rare_diseases/european_reference_networks/erf_en), are important initiatives to improve the systematic collection of evidence and provide a framework for the development of rational and improved treatments. In this review, we have tried to highlight controversial topics in the management of these disorders, which could inform the development of future clinical trials.

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DISCLOSURES

None.

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