

Adaptation in Gitelman Syndrome: “We Just Want to Pump You Up”*

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Most nephrologists will be confronted with cases of Gitelman syndrome (GS) during their careers. Although GS, hypokalemic alkalosis with hypomagnesemia and hypocalciuria, qualifies as a rare disease (<200,000 affected individuals in the United States), its US prevalence is estimated between 7000 and 10,000 cases (1), making it one of the most common inherited diseases of kidney tubules; in fact, the number of affected individuals likely exceeds the number of nephrologists practicing in this country (~6800). Furthermore, because GS often is detected during adolescence and adulthood, its diagnosis and treatment typically fall outside the purview of pediatric geneticists and in the realm of adult nephrologists. In this issue of the *CJASN*, Favre and colleagues (2) add important insights into processes that compensate for salt wasting in GS and that make the salt wasting phenotype so difficult to detect.

GS was first identified in 1966, when “three individuals [were] observed at the North Carolina Hospital with a syndrome characterized by hypokalemia, hypomagnesemia, alkalosis and clearly impaired conservation of magnesium and potassium.... Two of the patients [were] sisters. . .” (3). However, this syndrome was not deemed to be distinct from Bartter syndrome (BS) for a number of years, until it became clear that renal handling of calcium and magnesium (as well as clinical features) make GS and BS distinct (4). This insight shortly predated the molecular revolution, which permitted mutations in the thiazide-sensitive Na-Cl cotransporter (NCC; gene symbol *SLC12A3*) to be identified as causal, in most cases of GS (5). Coupled with the identification of mutations in the bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2; gene symbol *SLC12A1*) in BS (6), this insight confirmed the phenotypic analysis that the two syndromes represented two diseases. This simple dichotomy, however, quickly collapsed, as subsequent molecular and clinical analyses indicated, to the frustration of many medical students and nephrology fellows, that multiple gene defects can lead to several, sometimes overlapping, clinical syndromes of normotensive hypokalemia and alkalosis.

Recently, Seyberth (7) proposed a physiologic classification of salt-losing “tubulopathies” with secondary hyperaldosteronism (SLT), based on molecular genetics, combined with physiology and phenotype. According to this classification (modified in Table 1), SLTs comprise three predominant types: loop disorders, distal

convoluted tubule (DCT) disorders, and combined disorders. Classic GS, then, is one type of DCT disorder. This classification emphasizes that SLTs are genetic mimics of diuretic actions along the nephron. Accordingly, loop disorders, mimicking the effects of “high ceiling” loop diuretics, lead to polyuria, hypercalciuria, maternal polyhydramnios, and profound salt wasting during infancy. DCT disorders, mimicking the effects of thiazide diuretics, are milder, with hypocalciuria and hypomagnesemia accompanying the hypokalemic alkalosis. DCT disorders typically present after adolescence, with nonspecific symptoms; chondrocalcinosis, which is believed related to chronic hypomagnesemia, is increasingly recognized as a troubling manifestation (8–11). Finally, the combined disorders, mimicking combination diuretic treatment, as used commonly to treat resistant edema, are most severe, typically presenting before birth as maternal polyhydramnios and often leading to CKD.

When solute reabsorption is impaired along one nephron segment, either by diuretic drug treatment or genetic abnormality, other segments compensate, typically increasing reabsorption rates to compensate (partially) for solute rejected by the impaired segment (12). The mechanisms involved are complex and include responses to increased solute delivery (primary effects), responses to changes in extracellular fluid (ECF) volume (secondary effects), and structural changes (such as epithelial cell hypertrophy). Thus, inhibition of solute reabsorption along the thick ascending limb (TAL), as occurs in BS or during loop diuretic treatment, floods the distal nephron with NaCl, leading to increased reabsorption via NCC. The effect to increase NaCl reabsorption along the DCT is further enhanced when the renin/angiotensin/aldosterone pathway is stimulated by ECF volume depletion, because NCC is stimulated both by angiotensin II (13) and aldosterone (14). Finally, and often most importantly, DCT cells undergo hypertrophy, induced by increased solute delivery in the presence of neurohormonal stimulation; this hypertrophy is associated with increases in the abundance of transport proteins, such as NCC, and leads to further increases in NaCl transport capacity (15). These adaptations (represented schematically in Figure 1) are well studied in animals and in humans exposed to diuretic treatment. They appear to account for the remarkable potency of thiazide diuretics or metolazone to overcome resistance to loop diuretics. As might be expected, however, such treatment can

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Table 1. Classification of salt-losing tubulopathies with hyperaldosteronism					
Type of Disorder (Gene Product)	Traditional Name	Segment Affected	Pharmacotype	PH	Key Clinical Features
Loop disorders					
L1 (NKCC2)	BS I	Loop	Furosemide	+++	Polyuria, hypercalciuria, NC
L2 (ROMK)	BS II	Loop (+CD)	Furosemide (+ amiloride)	+++	Polyuria, hypercalciuria, NC, transient hyperkalemia
DCT disorders					
DC1 (NCC)	GS	DCT	Thiazide	–	Hypomagnesemia, hypocalciuria, growth retardation
DC2 (ClC-Kb)	BS III	DCT (+ TAL)	Thiazide (+ furosemide)	+	Hypochloremia, mild hypomagnesemia, FTT
DC3 (Kir 4.1)	EAST/SeSAME	DCT	Thiazide	–	Hypomagnesemia, hypocalciuria, EAST syndrome
Combined disorders					
L-DC1 (ClC-Ka/Kb)	BSDN	TAL + DCT	Furosemide + thiazide	+++	Polyuria, hypochloremia, mild hypomagnesemia, SND, CKD
L-DC2 (barttin)	BSDN	TAL + DCT	Furosemide + thiazide	+++	Polyuria, hypochloremia, mild hypomagnesemia, SND, CKD

All disorders have hypokalemia and alkalosis. The segment in parentheses is of secondary importance to the phenotype. PH; polyhydramnios; BS, Bartter syndrome; GS, Gitelman syndrome; BSDN, Bartter syndrome with sensorineural deafness; EAST/SeSAME, epilepsy, ataxia, sensorineural deafness, and tubulopathy; DCT, distal convoluted tubule; SND, sensorineural deafness; NC, nephrocalcinosis; FTT, failure to thrive; NCC, Na-Cl cotransporter; NKCC2, Na-K-2Cl cotransporter; ROMK, renal outer medullary potassium channel; ClC-Kb, chloride channel; Kir 4.1, inwardly rectifying potassium channel; NC, nephrocalcinosis.

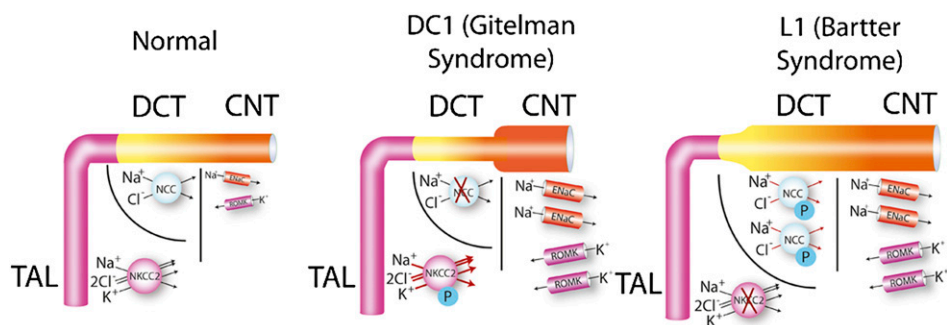


Figure 1. | Schematic representation of comparing transporter activity and nephron structure in normal individuals with individuals with DC1 (Gitelman syndrome [GS]) and L1 (Bartter syndrome [BS] type 1). GS results typically from inactivation of Na-Cl cotransporter (NCC). This floods the connecting tubule (CNT) with solute and leads to mild extracellular fluid volume depletion. These effects lead to hypertrophy of the CNT and activation of ENaC and the potassium channel, ROMK. As shown by Favre *et al.* (2), these individuals also have activation of Na-K-2Cl cotransporter (NKCC2), presumably via phosphorylation (P). In contrast, individuals with inactivation of NKCC2 flood the entire distal nephron with solute. These individuals develop hypertrophy throughout the distal nephron, with increases in the number and activity of salt transporting proteins.

lead to substantial hypokalemia and ECF volume depletion (16), as the adaptive processes are, in some ways, compensatory. In the case of transport inactivation along the TAL (loop diuretics or BS), much of the adaptive response is downstream; reabsorption is stimulated along segments exposed to increased solute delivery (17), although enhanced proximal reabsorption occurs as well.

The paper by Favre *et al.* in this issue of *CJASN* documents another form of nephron adaptation; in this case, nephron adaptation to the absence of NCC activity along the DCT (individuals with GS). Their studies used traditional clearance techniques during water diuresis and furosemide infusion to detect alterations in transport capacity by proximal

tubules and TALs. A surprising result is the failure to detect enhanced solute reabsorption along the proximal tubule, as has been detected in GS previously (18) and in mice with NCC knocked out (19). The results do, however, demonstrate enhanced solute reabsorption along the diluting segment, which typically includes both TAL and DCT; because such individuals lack functional NCC in the DCT, it is reasonable to infer that the TAL is the site of increased transport and that NKCC2 is the activated transporter.

The increased TAL reabsorption observed by Favre and colleagues suggests that activation of NKCC2 plays a role in limiting solute losses in GS patients. Although Loffing and colleagues (19) noted enhanced reabsorption along the

proximal tubule of NCC knockout mice, they did not emphasize an effect along the TAL, perhaps because they, and another group, could not detect an increase in NKCC2 abundance. However, it should be noted that delivery of NaCl to the distal tubule was lower in NCC knockout than in wild-type mice; this suggests that NKCC2 activity in the mouse model is increased, keeping distal delivery low. Although deleting NCC was not found to increase the abundance of NKCC2 (19,20), recent insights into NKCC2 regulation may provide a mechanism for the observations of Favre and colleagues. Cation chloride cotransporters, such as NCC and NKCC2, are functionally inactive until they are phosphorylated at key serine/threonine residues along their amino-terminal domains. Several groups have reported that the kinases SPAK (STE20/SPS1-related proline/alanine-rich kinase) and/or OSR1 (oxidative stress-responsive kinase 1) participate importantly in phosphorylating and activating NKCC2 (21). We recently found that depletion of the ECF volume altered the ratio of inhibitory to stimulatory SPAK, thereby increasing the abundance of phosphorylated NKCC2. We also found that NCC knockout caused the same SPAK switch and that this effect caused more activated NKCC2 to appear (22). Thus, mice that lack NCC activate (phosphorylate) NKCC2, an effect that should attenuate the solute losses that would otherwise occur.

The foregoing discussion raises a paradox about effects of NKCC2 and NCC deletion or inactivation. As highlighted in Figure 1, deleting NKCC2 leads to distal nephron hypertrophy together with increased NCC abundance. In contrast, deleting NCC does not cause TAL hypertrophy or increases in NKCC2 abundance (Figure 1); however, in both cases, transport activity is stimulated (23). A resolution to this paradox could derive from differential effects on solute delivery to the two nephron segments. NKCC2 deletion (or inactivation) greatly increases solute delivery to the DCT (and connecting tubule [CNT]), despite ECF volume contraction. This is a non-physiologic situation, in which transport is stimulated (by ECF volume depletion) when delivery is increased (in contrast, distal delivery is low when transport is stimulated physiologically by ECF depletion). Just like muscle work (“pumping iron”) stimulates muscle hypertrophy, epithelial cell solute throughput stimulates epithelial cell growth (23). Thus, epithelial hypertrophy only occurs when there is a combination of high solute delivery and transport stimulation. When NCC is deleted, transport activity is stimulated (perhaps by the SPAK switch noted above), but the ECF volume depletion and increased proximal NaCl reabsorption limit solute delivery to the TAL. In this situation, although transport capacity is enhanced (by phosphorylation and activation of the transporter), the increase is limited, and NKCC2 abundance and cellular size do not increase. In contrast, both NCC deletion and NKCC2 deletion (or inactivation) increase solute delivery to the CNT; thus, this segment is hypertrophic and hyperfunctional in both BS and GS (Figure 1). It is the increased CNT activity that leads to potassium wasting and alkalosis, which are characteristic of all of the SLTs.

The work by Favre and colleagues also suggests a novel insight into the hypocalciuria that is characteristic of GS. A running debate has posited roles of the proximal tubule and distal tubule in the hypocalciuria of GS and thiazide treatment. The interested reader is referred to a recent review by Reilly and Huang for a detailed analysis (both models are likely

correct; ref. 24), but it is rarely suggested that the TAL contributes importantly to this effect. If NCC deletion or inactivation activates NKCC2, as suggested by Favre and colleagues in humans, and by us in animals (22), this should increase the magnitude of the transepithelial voltage along the TAL. This voltage is the primary stimulus for paracellular calcium reabsorption along this segment, and an increase would tend to enhance calcium reabsorption. Accordingly, calcium reabsorption might be stimulated in individuals with GS all the way from the proximal tubule to the distal nephron; such stimulation is probably prerequisite to the remarkably low calcium excretion rates often observed in such individuals.

GS continues to vex nephrologists, both from the diagnostic and therapeutic standpoint. Recent work suggests that clinical testing has limited specificity (25), and we continue to need therapeutic approaches that are evidence-based. Sixteen years after its molecular basis was solved (5), we still have a lot to learn about this troubling, but fascinating, disorder.

Disclosures

None.

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*With apologies to “Hans and Franz.”

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See related article, “Localization of Tubular Adaptation to Renal Sodium Loss in Gitelman Syndrome,” on pages 472–478.