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NEPHROLOGY FORUM

Molecular pathogenesis of Bartter's and Gitelman's syndromes

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CASE PRESENTATION

A 42-year-old man presented to UCLA with a history of chronic hypokalemia and hypomagnesemia. His medical history was unremarkable except for occasional cramps in his calves. He had no history of laxative or diuretic abuse or of vomiting, and he was taking no medications. His 47-year-old brother also had a history of chronic hypokalemia and hypomagnesemia and severe intermittent muscle cramps. He was being treated with amiloride and magnesium supplementation. Both parents and a third brother were asymptomatic and had no electrolyte abnormalities.

Physical examination revealed a well-developed white male in no distress. His blood pressure was 110/70 mm Hg; heart rate, 72 beats/min; jugular venous pressure was estimated to be 7 cm; his chest was clear to auscultation. Cardiovascular examination disclosed a regular rate and rhythm, and no murmurs, rubs, or gallops; his abdomen was nontender without hepatosplenomegaly. Neurologic examination revealed cranial nerves 2-12 intact, no sensory or motor deficit, and no Chvostek or Trousseau sign. He did not have peripheral edema.

Laboratory values were: serum sodium, 136 mEq/liter; potassium, 2.4 mEq/liter; chloride, 94 mEq/liter; total CO_2, 28 mEq/liter; venous pH, 7.47 and PCO_2, 38 mm Hg; BUN, 10 mg/dl; serum creatinine, 0.7 mg/dl; ionized calcium, 1.25 mmol/liter; total calcium, 9.2 mg/dl; phosphorus, 3.2 mg/dl; magnesium, 1.1 mg/dl; supine plasma renin, 16.6 ng/ml/hr; supine aldosterone, 168 pg/ml; PTH, 13 pg/ml; $1,25(OH)_2$ vitamin D, 42 pg/ml. Urine chemistries were: sodium, 64 mEq/liter; potassium, 42 mEq/liter; chloride, 58 mEq/liter; calcium, 1 mg/dl; magnesium, 4.3 mg/dl; phosphorus, 67.4 mg/dl; and creatinine, 81 mg/dl. A diuretic screen was negative. The patient was discharged on no medications and has remained asymptomatic.

Key words: metabolic alkalosis, hyperprostaglandin E syndrome, hypokalemia, hypomagnesemia, hypocalciuria, nephrocalcinosis.

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DISCUSSION

DR. IRA KURTZ (*Chief, Division of Nephrology, Max Factor Professor of Medicine, Center for Health Sciences, UCLA School of Medicine, Los Angeles, California*): When Dr. Jay Stein presented a Nephrology Forum on Bartter's syndrome (BS) in this journal 13 years ago [1], he noted that "several questions remain to be clarified: Is Bartter's syndrome the consequence of a single defect, or rather the clinical phenotype of a variety of tubular defects? Is the syndrome caused by a defect in sodium chloride transport per se or by a primary potassium-wasting state? Are syndromes associated with magnesium and calcium loss, as well as potassium wasting, pathophysiologically linked?" These prescient questions can now be answered with more certainty because of the recent advances in our understanding of the molecular basis for Bartter's/Gitelman's syndromes.

The patient being discussed today presented with the following electrolyte abnormalities: hypokalemia with renal K^+ wasting (high fractional excretion); a chloride-resistant metabolic alkalosis, elevated plasma renin, elevated plasma aldosterone (relative to the serum K^+ concentration), hypomagnesemia with renal magnesium wasting (high fractional excretion), decreased urine calcium excretion, and normal blood pressure. These findings are compatible with either diuretic abuse due to thiazide-like diuretics or Gitelman's syndrome (GS). Because of the family history and the negative diuretic screen, a diagnosis of Gitelman's syndrome was made. In the absence of marked symptoms over many years, we elected not to treat the patient's electrolyte disturbances.

The patient under discussion has the classic features of GS [2, 3]. Patients with this disorder are normotensive and have a chloride-resistant metabolic alkalosis, increased plasma renin and aldosterone/K^+ ratio, hypokalemia, hypomagnesemia, and hypocalciuria. The syndrome was first described in 1966 by Gitelman et al in three patients, two of whom were sisters [2]. The metabolic abnormalities in Gitelman's syndrome shared many of the features of BS, a disorder first reported by Bartter et al four years earlier and with which it has subsequently been frequently confused in the literature [4]. Interestingly, the first case of BS might have been reported in 1957, when Rosenbaum and Hughes

Table 1. Clinical differences between Bartter's and Gitelman's syndromes

	Neonatal Bartter's syndrome	Adult Bartter's syndrome	Gitelman's syndrome
Polyhydramnios	+	−	−
Failure to thrive	+	−	−
Growth retardation	+	−	−
Polyuria	+	+	−
Polydipsia	+	+	−
Muscle cramps/spasm	−	−	±
Chondrocalcinosis	−	−	±

described an infant with hypokalemia, metabolic alkalosis, polyuria, growth failure, and recurrent episodes of volume depletion [5]. The patients reported by Bartter et al shared many of the features of GS in that they had a hypokalemic, chloride-resistant metabolic alkalosis, with elevated plasma renin levels and normal blood pressure. Unlike GS, the patients with BS had a urinary concentrating defect and growth failure and subsequently were shown to be hypercalciuric [6]. Despite these known differences, most clinicians fail to distinguish between these syndromes. Indeed, it appears that most patients reported in the literature with BS in fact have GS [7].

Clinical features

Bartter's and Gitelman's syndromes can be distinguished clinically (Table 1) as well as on the basis of differences in divalent cation metabolism [8–14]. In BS, symptoms are dictated by the patient's age at presentation. Polyuria and polydipsia are always present in children and adults with BS and can be the only presenting symptoms. Most cases of BS present in neonates [15–21]. Maternal polyhydramnios with premature labor is a common finding, presumably because of excessive urine production in utero [6, 21, 22]. Some of the infants have an appearance typical of BS, including a prominent forehead, triangular facies with drooping mouth, and large eyes and pinnae (Fig. 1) [23–25]. Subsequent difficulties in the neonate include failure to thrive and growth retardation [21]. Vomiting, diarrhea, and fever have been reported, and a variant of neonatal BS has been described with sensorineural deafness [25]. Neonatal BS can be diagnosed prenatally by finding elevated amniotic fluid chloride and aldosterone concentrations [26, 27].

Seyberth and colleagues have suggested that the severe neonatal form of BS be called the hyperprostaglandin E_2 syndrome [17, 28, 29]. Indeed, an increased rate of PGE_2 excretion is a common feature in BS [30–33]. Indomethacin has been used to decrease the incidence of polyhydramnios in pregnant patients with and without BS, although complications in the neonate, including acute renal failure, tricuspid regurgitation, and patent ductus arteriosus have been reported [19, 34–36]. In addition, indomethacin appears to improve the rate of growth in these children [34]. Given that the neonatal form seems to differ only in severity from the childhood and adult disorder, the term neonatal BS rather than hyperprostaglandin E_2 syndrome appears justified.

In contrast to BS, GS is a milder disease and doesn't present symptomatically in the neonatal period [7, 37]. Patients with GS are not volume depleted clinically. They can have a plasma aldosterone concentration that is not markedly elevated, as in the patient presented today, because their hypokalemia suppresses adrenal aldosterone production. Furthermore, patients with GS are often asymptomatic, or they only have neuromuscular complaints consisting of intermittent muscle spasms, cramps, or tetany [2, 3, 37–39]. Polyuria and polydipsia are not a feature of GS. In a subset of patients with electrolyte abnormalities indistinguishable from those of GS, arthritis in several joints due to chondrocalcinosis has been reported both in sporadic cases [40–43], and recently in a familial form [44].

Divalent cation disturbances in BS and GS

Several differences in divalent cation metabolism allow the two syndromes to be easily distinguished based on simple laboratory measurements (Fig. 2). Patients with BS have fasting hypercalciuria, whereas those with GS have a low urinary calcium excretion [6, 9–12, 14, 45]. In addition to hypercalciuria, medullary nephrocalcinosis is usually present in BS but is always absent in GS. The hypercalciuria in BS can predate the appearance of overt nephrocalcinosis. In addition to hypocalciuria, all patients with GS characteristically have hypomagnesemia and a high fractional excretion of magnesium [2, 10]. The patient presented today had a normal U_{mg}/U_{cr} of 0.05. In GS, urinary magnesium excretion reflects the dietary intake and only exceeds the normal excretion rate when patients are ingesting magnesium supplements [2, 10]. In BS, both the absolute urinary calcium excretion and the fractional excretion rate are increased. Despite the hypocalciuria in GS, the serum total and ionized calcium concentrations are normal, as in the patient presented today [10]. Colussi et al also reported that serum PTH levels are in the low normal range in GS, possibly because of the inhibitory effect of hypomagnesemia on PTH secretion [10]. In contrast, hypomagnesemia is not a typical finding in BS. A modest or transient decrease in serum magnesium has been reported in some neonates with BS. However, these infants generally are extremely ill, and whether the hypomagnesemia is due to extrarenal causes (vomiting, diarrhea) has not been excluded. Hypomagnesemia in adults diagnosed with BS in most cases is due to the failure to diagnose GS [9, 46]. Patients with BS and GS also differ in that the former have elevated $1,25(OH)_2D$ levels [8, 10, 12, 47] and PGE_2 excretion rates [30, 31, 33, 48]. The increased serum $1,25(OH)_2D$ in patients with BS occurs in the presence of normal 25-OHD, $24,25(OH)_2D$, PTH, and phosphorus levels [47]. The mechanism for elevated serum $1,25(OH)_2D$ levels in BS is

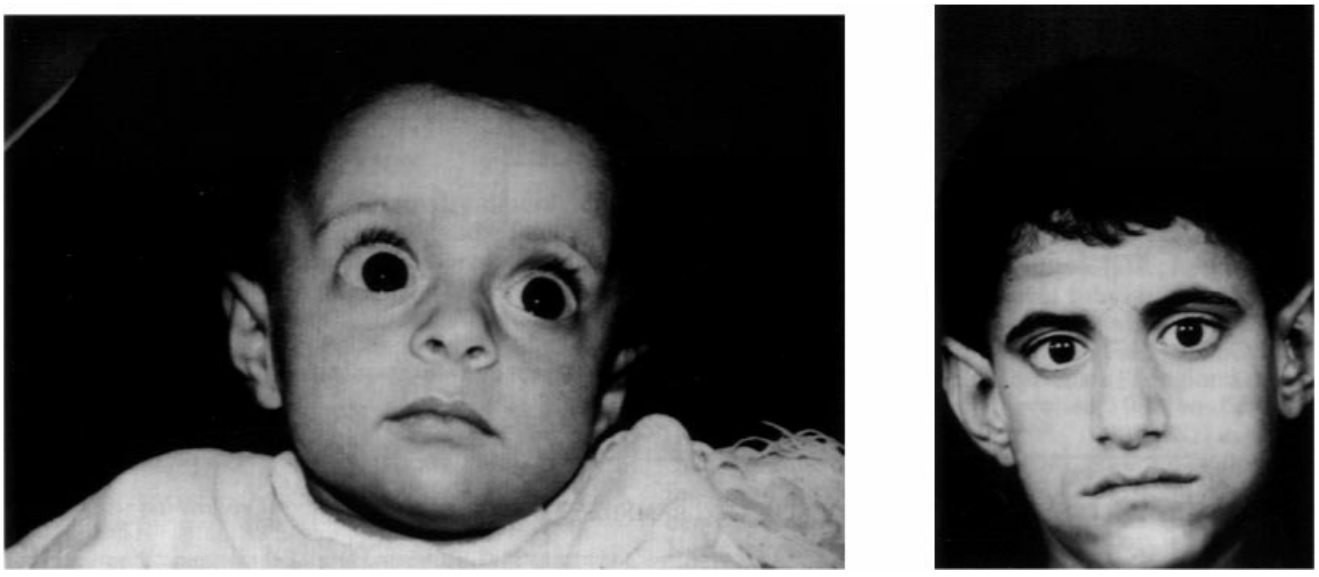

Fig. 1. Two patients with neonatal Bartter's syndrome described by Landau et al [25]. Both patients have the typical facial appearance with prominent forehead, triangular face, drooping mouth, and large eyes and pinnae.

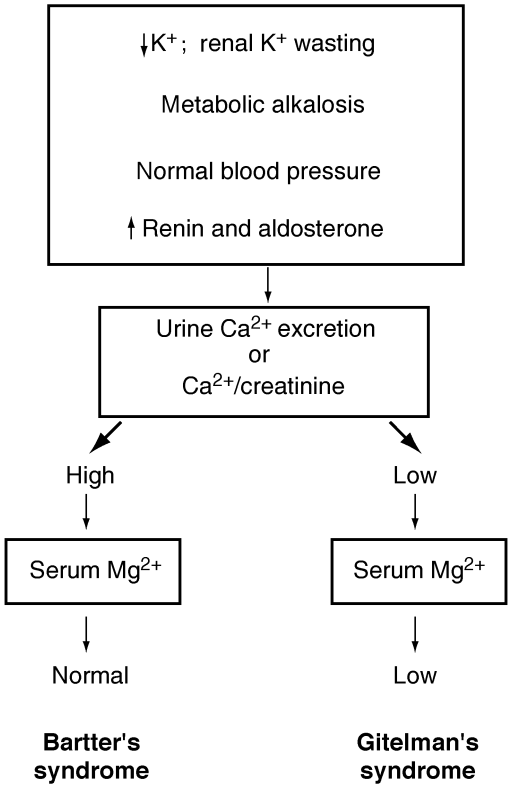

Fig. 2. Approach to the clinical differentiation between Bartter's syndrome and Gitelman's syndrome based on divalent cation metabolism. Note that some patients with type-3 BS have a normal urine Ca^{2+}/creatinine ratio.

unknown. Previous studies have shown that PGE_2 stimulates the hydroxylation of 25-(OH)D in thyroparathyroidectomized rats and in chick and rat kidney cells [49–51]. Restrepo de Rovetto et al have argued that PGE_2 stimulates the activity of 1α-hydroxylase, which results in elevated 1,25(OH) levels in BS [47]. Indomethacin therapy reduces the elevated $1,25(OH)_2D$ levels, Ca^{2+} excretion, and PGE_2 excretion in BS [8, 47]. A second unproven possibility is that the renal calcium leak per se increases plasma $1,25(OH)_2D$ levels.

Role of PGE in BS/GS

The BS/GS literature is replete with additional metabolic abnormalities that have contributed to the confusion regarding the pathogenesis of these syndromes. Most disputed has been the role of excess prostaglandin excretion in the pathogenesis of the electrolyte abnormalities [29–32, 52–56]. Indeed 17 years ago, a Nephrology Forum presented by Dr. Michael Dunn was devoted to the role of prostaglandins in BS [33]. Patients with GS usually have normal PGE_2 excretion rates [7, 57], although elevated excretion rates also have been reported in GS (that is, in patients misdiagnosed as having BS) [9, 58, 59]. Although cyclooxygenase inhibitors ameliorate some of the electrolyte disturbances and growth retardation in BS [34, 60], these drugs are less effective in GS [57, 61]. Excess PGE_2 excretion in BS is thought to be a secondary phenomenon [37]. This conclusion was reached prior to the description of the specific transport defects in BS/GS on the basis of the following considerations: (*1*) mepacrine, a potent phospholipase inhibitor, inhibits PGE_2 excretion without increasing plasma potassium and decreasing plasma renin [46]; (*2*) loop diuretics, which mimic several of the features of BS, increase PGE_2 production and/or excretion [62, 63]; and (*3*) hypokalemia is not a feature of prostaglandin-producing tumors [64].

Clinical assessment of the tubular transport defects in BS/GS

Early clearance studies suggested that the transport defect in BS was located in the diluting segment [65, 66]. Initially this finding was attributed to abnormal thick ascending limb function, although later studies suggested that abnormal NaCl transport in the distal convoluted tubule also resulted in abnormal urine diluting ability in some patients misdiagnosed with BS and in GS [9, 13, 61, 67–69]. The finding that patients with neonatal BS have a urine concentrating defect in addition to a diluting defect, fail to respond normally to furosemide, yet have a normal response to thiazides, suggested that a transport abnormality in the thick ascending limb of Henle was involved [70]. Similarly, the finding that patients with GS concentrate their urine normally, have hypocalciuria, fail to respond appropriately to thiazide diuretics, and have a greater-than-normal response to loop diuretics suggested that these patients have a transport abnormality in the distal convoluted tubule [9, 13, 61, 67–69, 71]. We know now that the mere presence of a diluting defect does not distinguish BS from GS, since both defective thick ascending limb and distal convoluted tubule NaCl transport lead to impaired urine diluting ability [61].

NaCl and calcium transport in the distal convoluted tubule

The characterization of the NaCl transport processes in the thick ascending limb of Henle (TAL) and the distal convoluted tubule (DCT) has proven invaluable in our understanding of the transport abnormalities in BS and GS. I will begin with a brief outline of normal DCT transport. The distal tubule comprises three distinct segments: the distal convoluted tubule, the connecting tubule, and the initial cortical collecting duct [72]. Most in-vivo data have been obtained from rats in which delineation among these segments is difficult. In-vivo transport studies, and more recent studies of immortalized mouse distal tubule cells, have shown that the DCT plays an important role in electroneutral NaCl absorption and PTH-responsive Ca^{2+} absorption [72, 73]. The molecular basis for electroneutral NaCl transport in the DCT was defined in 1993, when Gamba et al isolated a ~3.7 kb cDNA, flTSC (thiazide-sensitive cotransporter, also known as NCCT (NaCl cotransporter), that encoded the NaCl cotransporter in flounder (*Pseudopleuronectes americanus*) bladder [74]. A fragment of flTSC then was used to screen a rat renal cortical cDNA library; the rat NaCl cotransporter (rTSC1), a ~4.4 kb cDNA encoding a ~110 kD protein with 12 putative membrane-spanning domains, was isolated and identified [75]. Injection of oocytes with rTSC1 cRNA significantly increased Cl^--dependent, thiazide-inhibitable $^{22}Na^+$ uptake. In-situ hybridization and immunocytochemistry studies combined with functional data indicate that the NCCT gene (human gene mapping workshop approved symbol SLC12A3) encodes the apical membrane thiazide-sensitive NaCl cotransporter in the distal convoluted tubule [76–78]. In the rat kidney, polyclonal antibodies against rTSC1 intensely label the apical membranes in the distal nephron beginning in the early distal convoluted tubule and terminating within the connecting tubule; the intensity of labeling diminished from early to late sites along the distal tubule [78]. In addition to the DCT, evidence indicates NCCT expression in osteoblast-like cells [79] and peripheral blood mononuclear cells [80]. The physiologic role NCCT plays in these extrarenal sites remains to be determined.

We have known for three decades that thiazide diuretics decrease urinary calcium excretion [81]. Micropuncture and microperfusion experiments identified the distal convoluted tubule as the site where thiazides stimulate calcium absorption [82]. Stimulation of Ca^{2+} absorption has been attributed to (*1*) decreased apical Na^+ uptake driving basolateral Na^+/Ca^{2+} exchange with subsequent increased apical Ca^{2+} uptake; and (*2*) decreased intracellular chloride concentration, which causes hyperpolarization of the apical cell membrane and stimulates apical Ca^{2+} uptake via dihydropyridine-sensitive Ca^{2+} channels [82, 83]. Three alternatively spliced isoforms of the Na^+-Ca^{2+} exchange gene, NCX1–NACA2, NACA3, and NACA6–have been identified in these cells derived from the mouse DCT, which might mediate Na^+-dependent Ca^{2+} efflux from the DCT [84]. The relative role of the basolateral Na^+/Ca^{2+} exchange isoforms and Ca^{2+}-ATPase in mediating cellular calcium extrusion is currently unknown; both transporters are co-expressed with rTSC1 in the late DCT and connecting tubule [78].

Molecular basis for Gitelman's syndrome

It was an "annum mirabilis" for this field in 1996, when Lifton's group uncovered the molecular basis for GS [85] and BS [86, 87] and allowed many of Dr. Stein's questions to be answered. Simon et al first demonstrated complete linkage of GS to the NCCT gene locus (SLC12A3) on chromosome 16q13, and specified GS as an autosomal recessive disorder with 99% penetrance [85]. The linkage to chromosome 16q13 has been confirmed by others [88–91]. Simon et al identified a wide variety of mutations distributed throughout the NCCT gene consistent with loss-of-function alleles. Additional potential loss-of-function mutations also were reported in 1996 [90, 92]. Missense mutations are most frequent and are usually localized to the intracellular domains of the protein rather than to the extracellular or transmembrane domains. Many of the patients described thus far are compound heterozygotes. This finding, together with the documentation of independent mutant alleles within the same kindred, suggests that mutant alleles are not rare. In the Swedish and Italian populations studied, the prevalence of heterozygotes based

Normal	GVMIRCMLNI	WGVILYLRLP	WITAQAGIVL	TWIIILLSVT	VTSITGLSIS	190
F	GVMIRCMLNI	WGVILYLRLP	WITAQAGIDE	KTEAQRREMN	VGRMVNE	187
M	GVMIRCMLNI	WGVILYLRLP	WITAQAGIVL	TWIIILLSVT	VTSITGLSIS	190

Normal	MDQERKAIIS	LLSKFELGFH	EVHILPDINQ	NPRAEHTKRF	EDMIAPFRLN	930
F	----------	----------	----------	----------	----------	187
M	MDQERKAIIS	LLSKFELGFH	EVHILPDINQ	NPRAEHPFGR	----------	920

Fig. 3. Predicted amino acid sequence of paternal (F) and maternal (M) NCCT polypeptides in the presented patient with GS. Messenger RNA was isolated from peripheral blood mononuclear cells obtained from normals and from the patient under discussion with GS. Following reverse transcription with AMV reverse transcriptase, NCCT cDNA was amplified by PCR and sequenced. Genomic DNA from the patient and his parents also was sequenced to determine the inheritance of each allele. Two different mutations were detected in the patient's NCCT cDNA (compound heterozygote). The cDNA from the patient's paternal allele had an additional 119 bp insertion between exon 3 and 4, generating a predicted premature stop codon after amino acid 187. In cDNA from the maternal allele, exon 24 was deleted, resulting in a predicted premature stop codon after amino acid 920.

on phenotypic expression is approximately 1% [14, 85, 93]. Simon et al have estimated a sporadic prevalence of 0.001 and a mutant allele frequency of 1/200 [85].

The patient presented today is a compound heterozygote (Fig. 3). Two different mutations were detected in the patient's NCCT cDNA obtained from his peripheral blood mononuclear cells. In cDNA derived from the patient's maternal allele, exon 24 was deleted and resulted in a premature stop codon (after amino acid 920). The cDNA derived from the paternal allele had an additional 119 bp insertion between exon 3 and exon 4, generating a premature stop codon (after amino acid 187). The patient's genomic DNA had a previously reported 5′ splice mutation in intron 24, GGT → GTT maternal allele) [85], and a previously unreported 3′ splice site mutation in intron 3, CAG → CAA (paternal allele), which resulted in the activation of a nearby cryptic splice site in intron 3 [80].

The severity of symptoms has varied widely in families with Gitelman's syndrome. The phenotypic consequences of the mutations described thus far are unknown. Whether patients with homozygous frameshift mutations have a more severe phenotype than do those with homozygous or compound heterozygous missense mutations remains to be determined [92]. Interestingly, the patient under discussion was essentially asymptomatic, whereas his brother, who has identical mutations in the NCCT gene, had frequent muscle cramps and spasms. The electrolyte abnormalities in the brother were essentially the same, that is, serum sodium, 140 mEq/liter; potassium, 2.3 mEq/liter; chloride, 94 mEq/liter; total CO_2, 27 mEq/liter; BUN, 13 mg/dl; serum creatinine, 1.0 mg/dl; ionized calcium, 1.21 mmol/liter; total calcium, 9.1 mg/dl; phosphorus, 3.7 mg/dl; and magnesium, 1.1 mg/dl. These findings suggest that other biochemical abnormalities or psychogenic or environmental factors are modifying the severity of the symptoms in the two brothers. Alternatively, a background gene effect could modify the electrolyte abnormalities and/or symptoms in patients with identical mutations in the NCCT gene locus. The creation of knockout mice with various NCCT mutations will be required to correlate specific mutations with the severity of specific GS phenotypes (biochemical abnormalities, symptoms). In addition, comparing identical mutations in several strains of mice will help determine the role of background genes in altering the phenotypic severity in GS.

In 1979, Bauer et al reported a syndrome with electrolyte abnormalities identical to GS but which differs from the classic disorder in that patients have arthritis due to chondrocalcinosis [40]. As I mentioned earlier, several sporadic cases have since been described [41–43, 94]. Smilde and colleagues reported a familial form of the disorder in which 7 affected family members had radiologic abnormalities involving their wrists, elbows, shoulders, knees, and symphysis pubis [44]. Magnesium therapy appeared to improve the radiologic appearance of the joint abnormalities in one patient over a 10-year followup. Magnesium, a known cofactor in the conversion of pyrophosphate to inorganic phosphate, increases the solubility of calcium pyrophosphate in vitro [95]. However, if hypomagnesemia is causally related to the arthritis in these patients, it is unclear why most patients with GS and patients with other hypomagnesemic disorders don't have chondrocalcinosis. Whether a separate gene locus accounts for this syndrome or whether background genes alter the typical GS phenotype is unknown. The gene for a separate

familial form of chondrocalcinosis associated with intermittent benign seizures and lacking the metabolic abnormalities in GS has been localized to chromosome 5p [96].

Na-K-2Cl cotransport

Clearance studies suggested that the thick ascending limb (loop of Henle) was the site of the transport abnormalities in BS [65]. The characteristics of Na^+, K^+, and Cl^- transport in the cortical thick ascending limb (cTAL) and medullary thick ascending limb (mTAL) were first demonstrated by Rocha and Kokko [97] and by Burg and Green [98]. Transport studies in intact nephrons and vesicle preparations have established that the predominant mode of apical Cl^- absorption is via a furosemide-sensitive Na-K-2Cl cotransporter [99]. Sodium chloride transport in the TAL is stimulated by hormones that activate cAMP, and is inhibited by extracellular Ca^{2+}, PGE_2, ANF, and cytochrome P-450 arachidonate metabolites [100]. In 1994, using a homology-based screening strategy and functional expression in oocytes, Gamba et al reported the isolation of a ~4.7 kb cDNA encoding the rat apical Na-K-2Cl (NKCC2) cotransporter [75]. Payne and Forbush reported several splice variants in rabbit and mouse kidney, which they have called NKCC2A, NKCC2B, and NKCC2F [101]. Using PCR, Yang et al have identified a fourth isoform, NKCC2AF, in rat kidney [102]. These isoforms, which arise from alternative splicing of cassette exons, result in a variable 96-bp region located in the second transmembrane domain. Amplification by PCR of DNA derived from specific nephron segments revealed segment-specific expression: the A isoform is expressed in the cTAL, mTAL, and macula densa; B isoform is expressed in the cTAL and macula densa; F isoform is expressed in the mTAL and outer medullary collecting duct (OMCD); and AF is expressed in the mTAL. Whether these splice variants differ in their functional characteristics is unknown. Immunoblot studies show that the major NKCC2 protein is approximately 150 kD [103]. The topology of this transporter is similar to NCCT in that both proteins have 12 putative membrane spanning domains and a central hydrophobic core region. The overall sequence identity between the transporters is approximately 50%. Immunocytochemistry studies have localized the NKCC2 protein to the apical membrane of the cTAL and mTAL [103]. Other members of the Na-K-2Cl cotransport family have been cloned, including the secretory form of the Na-K-2Cl cotransporter, NKCCl, first cloned from dogfish shark rectal gland [104], and subsequently cloned from human colonic cells [105] and murine inner medullary collecting duct [106]. More recently, the KCl cotransporter KCCl encoding a 1085 amino acid polypeptide has been cloned [107]. The KCCl cotransporter shares approximately 25% homology with other members of the cation-chloride cotransporter family, is expressed in several tissues, and might represent the housekeeping form responsible for cellular volume regulation. In the renal tubule, this transporter might play a role in basolateral KCl efflux in the rabbit proximal tubule [108] and cTAL [109].

Mechanism for K^+ secretion in the TAL and CCD

Low conductance (30 pS) inward rectifying K^+ channels play a significant role in mediating K^+ secretion in the thick ascending limb (TAL), the cortical collecting duct (CCD), and various tissues [110]. Luminal K^+ efflux in the TAL ensures an adequate supply of K^+ for the apical Na-K-2Cl cotransporter. In the CCD, K^+ secretion by principal cells ensures adequate renal excretion of dietary K^+ intake. Efforts at identifying channel molecules responsible for renal K^+ secretion resulted in the expression cloning of ROMK1 (rat outer medulla K^+ channel) from rat renal outer medulla [111], and subsequently in the cloning of several splice variants in rats (ROMK1-3,6 [112]) and humans (ROMK1-5 [113]). This divergence of ROMK mRNA results in the formation of channels with variable lengths of amino termini. The ROMK channels are members of a superfamily of structurally and functionally related K^+ channel proteins [110]. The common functional properties shared by these channels is their inwardly rectifying current voltage relationship. Rectification can be weak or strong and is due to a voltage-dependent block of the channel pore by intracellular magnesium and polyamines. The ROMK channels expressed in *Xenopus* oocytes form a weakly inwardly rectifying potassium-channel current with a single channel conductance of 35–45 pS [114]. The channel requires magnesium and ATP to open, exhibits a high open probability, and is inhibited by millimolar concentrations of ATP, low intracellular pH, and arachidonic acid [110, 114]. These properties are comparable to those of the low-conductance potassium channel electrophysiologically identified in the apical membrane of thick ascending limb and cortical collecting duct cells [110]. In rats, ROMK1 transcripts are expressed in the CCD, OMCD, and inner medullary collecting ducts; ROMK2 is located in the mTAL, cTAL, DCT, connecting tubule (CNT), and CCD; and ROMK3 is found in the mTAL, cTAL, DCT, and CNT [115, 116]. No information exists regarding isoform distribution in the human nephron. No functional differences have been found thus far in human ROMK1-5 when expressed in *Xenopus* oocytes [117]. The ROMK2 potassium channel and the cystic fibrosis transmembrane regulator (CFTR) can form a complex, and co-expression of ROMK2 and CFTR increases the sensitivity of ROMK2 to the K^+ channel inhibitor glibenclamide [118]. However, it has been suggested that the ROMK6 isoform is more likely to associate with CFTR and form the epithelial ATP-sensitive potassium channel, at least in the rat [112].

Table 2. Genetic differences between Bartter's and Gitelman's syndromes

Clinical syndrome	Gene locus	Chromosome
Bartter's syndrome		
Type-1	SLC12A1	15q15-q21
Type-2	KCNJ1	11q24
Type-3	CLCNKB	1p36
Gitelman's syndrome	SLC12A3	16q13

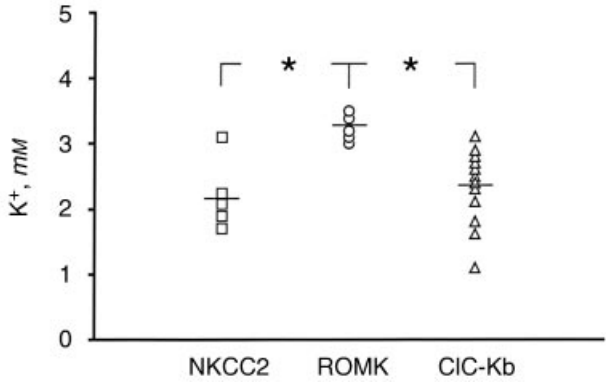

Fig. 4. The severity of hypokalemia in type-1 (NKCC2, □) versus type-2 (ROMK, ○) and type-3 (ClC-Kb, △) Bartter's syndrome mutations. Mean serum potassium in type-1 BS was 2.2 ± 0.24 mEq/liter, 3.3 ± 0.08 mEq/liter in type-2 BS, and 2.4 ± 0.12 mEq/liter in type-3 BS. The data in patients with ROMK mutations were obtained from Derst et al [117]. The data in patients with NKCC2 mutations came from Simon et al [86]. The data in patients with ClC-Kb mutations were from Ref. 128. * $P < 0.001$.

Mechanism for basolateral Cl^- transport in the TAL

Chloride is absorbed across the basolateral membrane of the TAL in part via chloride channels [119]. Three structural classes of chloride channels have been described: (*1*) CFTR (cystic fibrosis transmembrane regulator); (*2*) glycine and $GABA_A$ receptors; and (*3*) the CLC family of Cl^- channels [120]. It is the last group of chloride channels that plays an important role in mediating basolateral Cl^- transport in the TAL. Jentsch et al identified the first of the voltage-gated CLC genes (C1C-0) by expression cloning from the marine ray *Torpedo marmorata* [121]. Nine CLC family members (ClC-1 through ClC-7, ClC-Ka and ClC-Kb) are known to play a role in transport, volume regulation, and muscle and neuronal excitability in many tissues. ClC-Ka and ClC-Kb are expressed exclusively in the human kidney [122, 123]. Expression of ClC-Ka, ClC-Kb, and the rat homologues ClC-K1 and ClC-K2 has been localized by RT-CR in various nephron segments. The precise localization throughout the nephron of these ClC chloride channels at the protein level is currently unknown [122, 124, 125].

Molecular basis for Bartter's syndrome: mutations in NKCC2, ROMK, and ClC-Kb

Simon et al demonstrated linkage to the NKCC2 gene locus (SLC12A1) in 9 patients from five families with neonatal BS [86]. The authors mapped the gene to chromosome 15q15-q21 using a (GT)n dinucleotide repeat sequence. The NKCC2 protein is encoded by 26 exons, with the coding region spanning 80 kb of genomic DNA. Six mutations in NKCC2 were identified, including non-conservative missense and frameshift mutations. In the five families studied, all affected offspring were homozygous for alleles of markers used for genotyping. In four of the five families, affected individuals were offspring of a consanguineous union. The latter finding differs in patients with GS, who are most often compound heterozygotes and offspring of a non-consanguineous union, as was the patient discussed today. A possible explanation for the difference is that heterozygotes in the population with NCCT mutations are more common. We now refer to patients with BS due to NKCC2 mutations as having type-1 BS.

The genetic heterogeneity of neonatal BS was demonstrated recently (Table 2) [87, 126]. Some patients with neonatal BS have mutations of the ROMK potassium-channel gene locus (KCNJ1) on chromosome 11q24. Non-conservative missense mutations, frameshift mutations, and mutations predicted to cause premature truncation of the protein were identified. We refer to patients with ROMK mutations as having type-2 BS. As I said earlier, 5 ROMK splice variants have been identified in human kidney, although their relative distribution in the distal portion of the nephron is unknown. All 5 isoforms have a common 372 amino acid core encoded by exon 5. The mutations described thus far affect exon 5 and involve all ROMK isoforms. Derst and colleagues analyzed the channel activity of 5 ROMK channel mutations by mutating wild-type rat ROMK1 transfected into COS-7 cells [117]. In cells expressing mutated ROMK1, channel currents were low to absent. Mutations in the N- or C-terminus had low but significant channel currents.

Given the importance of ROMK channel activity in mediating both K^+ recycling in the TAL and K^+ secretion in the CCD, one might expect that hypokalemia would be less severe in patients with BS who have ROMK mutations. Impaired K^+ secretion by the CCD would be predicted to ameliorate the K^+ wasting that occurs because of impaired TAL function. Furthermore, complete loss of ROMK function might not affect TAL transport to the same extent as mutations causing complete loss of function of NKCC2, because of the presence of 70 ps K^+ channels in the TAL, which mediate approximately 80% of the apical K^+ conductance under control conditions [110]. This channel might partially restore K^+ recycling in patients with ROMK defects. A comparison of patients with BS with NKCC2 mutations (type-1 BS) versus ROMK defects (type-2 BS) indicates that the difference in the degree of hypokalemia is an important distinguishing clinical feature in some patients (Fig. 4). In addition, it would be expected that patients with type-2 BS would have a lower transtubular

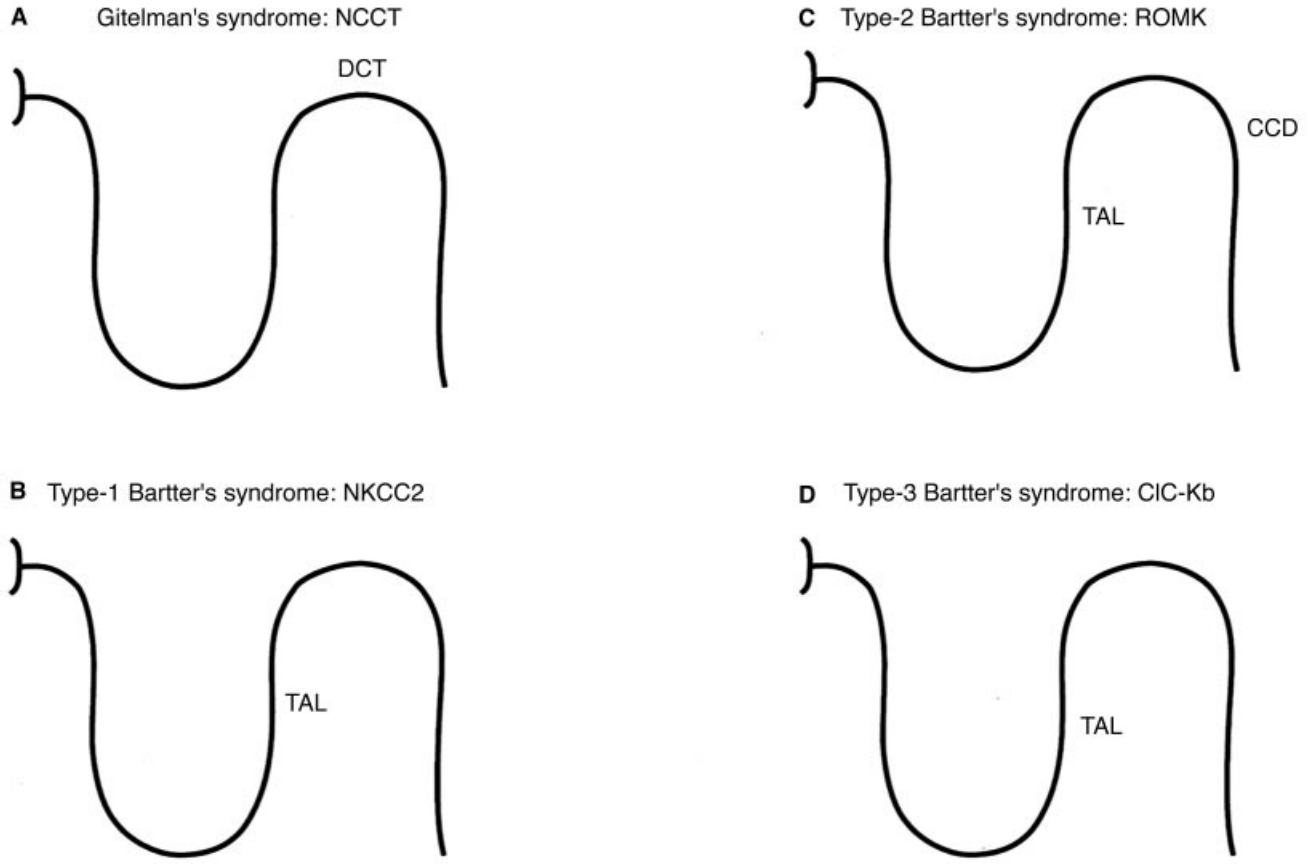

Fig. 5. Localization of transport abnormalities in Gitelman's and Bartter's syndromes.

potassium gradient (TTKG) and less of a decrease in urinary K^+ excretion in response to amiloride administration. However, not all patients with ROMK mutations have mild hypokalemia [87]. In a study in rats by Wang et al, the K^+ channel blocker U37883A inhibited Na^+ and K^+ absorption in the TAL and K^+ secretion in the CCD; a natriuresis ensued without a change in urinary K^+ excretion [127]. This could be an interesting model for studying the effect of certain lack-of-function ROMK mutations on blood and urine chemistries prior to the availability of ROMK knockout mice.

Simon et al recently reported that mutations in the CLCNKB gene locus on chromosome 1p36 encoding the ClC-Kb protein also lead to a BS phenotype (type-3 BS) [128]. Eleven different mutations including large deletions and missense and nonsense mutations were characterized in 17 kindreds. As shown in Figure 3, patients with type-3 BS have more severe hypokalemia than do patients with type-2 BS, and they have hypokalemia comparable to that in patients with type-1 BS. Interestingly, none of the patients with type-3 BS had nephrocalcinosis, and 6 of the 17 index cases had urine Ca^{2+}/creatinine ratios in the normal range. The absence of nephrocalcinosis distinguishes these patients from those with NKCC2 or ROMK defects. Whether patients with CLCNKB mutations have microscopic nephrocalcinosis detectable by renal biopsy remains to be determined. Of interest is the discovery that mutations in another member of the CLC family, CLCN5, causes X-linked hypercalciuric nephrolithiasis (XLHN) [129]. Unlike type-3 BS, these patients have nephrocalcinosis. The localization of the transport abnormalities in GS and BS is depicted in Figure 5.

Related uncharacterized syndromes

The literature contains reports of several patients with normotensive hypokalemic metabolic alkalosis but additional features that prevent them from being classified as having GS or BS. These disorders likely are due to different renal tubular transport defects. Runeberg and coworkers described a 6-year-old boy with tetany and arthritis in the knees and ankles [130]. He had hypokalemia, metabolic alkalosis, normal blood pressure, hypocalcemia, hypercalciuria, a high fractional excretion of magnesium, chondrocalcinosis, and nephrocalcinosis. Renal biopsy showed juxtaglomerular cell hyperplasia and interstitial fibrosis. Oral magnesium therapy slowed the progression of the nephrocalcinosis and decreased the intra-articular calcifications. Güllner et al reported 3 siblings, ages 10 to 14 years, who

presented with mild growth retardation, hypokalemia, metabolic alkalosis, normal blood pressure, elevated plasma renin activity, and resistance to angiotensin II [131, 132]. The serum magnesium was normal (1.51 ± 0.06 mEq/liter). One child had polyuria, polydipsia, and neuromuscular complaints, whereas the others were asymptomatic. The PGE_2 excretion was elevated in all the patients. The urinary calcium excretion was not measured. Clearance studies revealed normal distal fractional Cl^- absorption, and the renal biopsy showed interstitial fibrosis and abnormalities restricted to proximal tubules without juxtaglomerular cell hyperplasia. Mehrotra et al recently described a 44-year-old man with hypokalemia, metabolic alkalosis, normal blood pressure, mild renal insufficiency, hypermagnesemia, normocalcemia, a low fractional excretion of magnesium, hypocalciuria, glucosuria, and elevated plasma renin activity and plasma aldosterone levels [133]. Clearance studies showed a high fractional delivery of Cl^- to the diluting segment and a high distal fractional reabsorption of Cl^-. The patient's appropriate response to furosemide and chlorothiazide administration suggested intact TAL and DCT function. A renal biopsy was not performed; however, a clinical diagnosis of chronic interstitial nephritis was made. The authors postulated a defect in proximal tubule NaCl absorption. Williams et al reported a neonate whose prenatal course was complicated by polyhydramnios [18]. The infant, who was volume depleted, required mechanical ventilation and developed generalized seizures at 6 days of age, had hypokalemia, hypomagnesemia, metabolic alkalosis, hypophosphatemia with renal phosphate wasting, polyuria, hypercalciuria, hypermagnesuria, glucosuria, severe hyponatremia, and adrenal insufficiency. Nephrocalcinosis was not present. The infant died at 4 months and autopsy revealed juxtaglomerular cell hyperplasia and extensive tubular dilation (primarily of the proximal tubules) with poorly developed brush borders. The authors suggested that a proximal tubule defect could account for the electrolyte abnormalities. It is of interest that despite a proposed defect in proximal tubule transport, bicarbonate absorption in this segment appears to have been spared, given that the patients are alkalemic rather than having proximal renal tubular acidosis. Whether mutations in KCC1 are responsible for any of these uncharacterized syndromes with abnormal proximal tubule transport is unknown.

Summary of diagnostic considerations

As I said, patients with Gitelman's and Bartter's syndromes differ in their clinical presentation and laboratory findings (Table 1, Fig. 2). Patients with BS usually present as neonates; patients with GS present in their teens or as adults. Second, the predominant symptoms in BS are polyuria and polydipsia; patients with GS usually complain of muscle spasm and cramps. The degree of hypokalemia is equally as severe in patients with GS and those with type-1 BS (NKCC2 defect) and type-3 BS (ClC-Kb defect). Patients with type-2 BS (ROMK defect) can have less severe hypokalemia. Differences in divalent cation metabolism clearly distinguish GS from BS. Patients with GS are hypomagnesemic and hypocalciuric. In contrast, patients with BS usually have a normal serum magnesium, are hypercalciuric, and develop nephrocalcinosis. Interestingly, patients with type-3 BS fail to develop nephrocalcinosis and are not uniformly hypercalciuric.

In summary, loss of function mutations in NCCT causes GS. In this disorder, there is defective NaCl transport in the distal convoluted tubule. Patients with BS (type-1, type-2, and type-3) have loss of function mutations in the transporters that are required for the normal absorption of Na^+, K^+, and Cl^- in the TAL (NKCC2, ROMK, and ClC-Kb).

QUESTIONS AND ANSWERS

Dr. Nicolaos E. Madias (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): Could you tell us whether any of the mutated proteins of the Na-K-2Cl cotransporter or the NaCl cotransporter have been expressed in cell systems, and whether the in-vitro findings correlate with disturbed function?

Dr. Kurtz: At present, no studies have addressed the effect of specific mutations in type-1 BS on NKCC2 function in vitro. Derst et al have analyzed five recently described ROMK channel mutations causing type-2 BS [117]. The authors expressed mutated rat ROMK1 in COS-7 cells and were able to demonstrate the effect of specific type-2 BS mutations on channel currents. Whether these in-vitro findings will correlate with a particular clinical phenotype is unknown. The number of patients studied to date is too small to determine whether the severity of polyuria, hypokalemia, and hypercalciuria/nephrocalcinosis correlates with specific mutations. It is becoming increasingly evident that background genes also can alter a given phenotype, so it might be overly simplistic of us to expect a close correlation between a specific mutation and the clinical severity of BS in all patients [134–136]. With regard to GS, Kunchaparty et al have recently expressed in *Xenopus* oocytes the mouse NaCl cotransporter incorporating a GS mutation (premature stop codon at amino acid 968), which abolished function [137]. In addition recently we showed that peripheral blood mononuclear cells (obtained from the patient with GS discussed today) lack chlorothiazide-inhibitable ^{22}Na uptake [80]. Our further understanding of the physiologic processes in the kidney that result in the clinical differences between these disorders likely will depend on the creation of knockout mice with GS and BS phenotypes. This approach also will allow the role of background genes and several other candidate genes to be tested.

Dr. Madias: Could you summarize for us the current

understanding of genotypic-phenotypic relationships in these disorders? To what do you attribute the phenotypic variability between patients with these syndromes? Also, could you address the abnormalities of heterozygote individuals?

Dr. Kurtz: Mutations in the SLC12A3 gene locus are the only known cause of Gitelman's syndrome. Whether patients with chondrocalcinosis and arthritis as well as with the electrolyte abnormalities found in GS have SLC12A3 mutations will require further studies. In Bartter's syndrome, because three genes are known to be involved, one might expect a wider spectrum of clinical and laboratory abnormalities. In addition, no patients with GS have been found to have NKCC2, ROMK2, or ClC-Kb defects. Conversely, no patients with the clinical features of BS have NCCT mutations.

You raise the interesting issue of heterozygotes. One might speculate that these individuals have subtle but detectable salt wasting and potassium wasting, which generally is not apparent clinically. It is unknown, for example, whether in either syndrome heterozygotes have more negative salt balance when placed on a zero Na^+ intake, and more negative potassium balance when placed on a zero K^+ intake. Studies addressing these issues would be of interest.

Dr. Madias: It would be interesting to see in expression studies whether a mutated protein might cause increase of function.

Dr. Kurtz: It is certainly possible, although as yet unproven, that certain patients with hypertension have gain-of-function mutations. This issue is currently an active area of investigation. Virtually all transport proteins known to mediate Na^+ transport directly or indirectly in the kidney are being studied as candidate genes for gain-of-function mutations in patients with idiopathic hypertension.

Dr. David Lee (*Chief, Division of Nephrology, Sepulveda Veterans Administration Hospital, and Professor of Medicine, UCLA, Los Angeles, California*): Could you expand on the role that prostaglandins play in Bartter's syndrome? The neonatal form of Bartter's syndrome is sometimes called hyperprostaglandin E syndrome. In addition, do all patients respond to indomethacin, or only a subgroup?

Dr. Kurtz: The role of prostaglandins in BS is controversial. Lüthy and colleagues have suggested that, in most patients with Gitelman's syndrome, prostaglandin E_2 excretion is normal and indomethacin is less effective in correcting the hypokalemia [57]. However, some patients originally diagnosed with BS in retrospect actually had GS; they had abnormal prostaglandin excretion and electrolyte abnormalities that were ameliorated by indomethacin [46, 67]. In BS, PGE_2 excretion usually is increased. As I mentioned, Seyberth et al suggested that the neonatal form of the disorder be called the hyperprostaglandin E syndrome [29]. Cyclo-oxygenase inhibitors do ameliorate many of the features of BS, although the electrolyte abnormalities are not completely normalized. Excess PGE_2 excretion in BS can directly inhibit Na-K-2Cl cotransport [100, 138], and arachidonic acid inhibits ROMK1 channels [139]. These effects would be predicted to exacerbate the underlying transport abnormalities in BS. Furthermore, as I discussed, PGE_2 might play a role in increasing $1,25(OH)_2$-vitamin D in BS, thereby possibly contributing to the hypercalciuria and nephrocalcinosis [47]. On the other hand, Delaney et al have shown that mepacrine, a phospholipase inhibitor, normalized PGE_2 excretion in their patients without altering body weight or plasma potassium [46]. This finding argues against a role for prostaglandins. Furthermore, Zusman et al reported a patient with a prostaglandin-producing renal cell carcinoma who did not have the electrolyte abnormalities of BS [64].

Dr. Madias: You referred to other syndromes with high plasma prostaglandin levels. Normal pregnancy is such a situation. High prostaglandin levels are associated with elevated plasma renin and aldosterone, hyporesponsiveness to vasoconstrictors, and low urinary calcium excretion, that is, a physiologic model of GS.

Dr. Kurtz: The high prostaglandin levels you describe would be characteristic of BS, whereas hypocalciuria is indeed reminiscent of GS. It is of interest that when hypocalciuria occurs in pregnancy, it is usually in the context of pre-eclampsia [140]. The mechanism does not appear to be decreased intestinal uptake, and likely it is mediated by the kidney [141]. Furthermore, in pre-eclampsia, the hypocalciuria is associated with low $1,25(OH)_2$-vitamin D levels [142]. Despite elevated urinary aldosterone excretion in pregnancy [143], hypokalemia, hypomagnesemia, and a chloride-sensitive metabolic alkalosis rarely are present.

Dr. Charles Kleeman (*Attending Physician, West Los Angeles Veterans Administration Hospital, and Professor of Medicine, UCLA*): What drives the hypereninemic hyperaldosteronemic state? What is the fundamental cause of this state in both GS and BS?

Dr. Kurtz: It is currently believed that the elevated plasma renin activity is due to various degrees of volume depletion resulting from defective renal NaCl absorption. This scenario is analogous to that seen in patients taking diuretics, that is, mild volume depletion in GS and severe volume depletion in neonatal BS. In many patients, however, the aldosterone level is not elevated because of the suppressive effect of concomitant hypokalemia on adrenal aldosterone production.

Dr. Madias: Is the ROMK channel expressed outside of kidney, and are ROMK mutations associated with pathology elsewhere?

Dr. Kurtz: By Northern analysis, ROMK message has been detected only in the kidney [113]. However, with more

sensitive PCR techniques, one can detect ROMK isoforms in various extrarenal tissues [112, 113]. We do not know whether functional abnormalities in various ROMK channels outside the kidney play a role in the manifestations of type-2 BS.

Dr. Lee: You mentioned that the thiazide-sensitive NaCl cotransporter is located in the distal tubule. Were the results you reported regarding expression of this transporter in lymphocytes performed at the protein level?

Dr. Kurtz: Thus far we have shown by Northern analysis that a 4.4 kb transcript of the thiazide-sensitive NaCl cotransporter is present in human peripheral blood mononuclear cells [80]. We also recently demonstrated an absence of thiazide-inhibitable Na^+ transport in cells obtained from 2 patients with GS [80]. In regards to Dr. Madias' previous question regarding extrarenal ROMK expression, one could speculate that the dermatitis reported in some patients with GS [2] is somehow related to abnormal lymphocyte NaCl cotransport.

Dr. Arnold Felsenfeld (*Attending Physician, West Los Angeles Veterans Administration Hospital, Professor of Medicine, UCLA*): Dr. Kurtz, you made the analogy between the loop diuretics and BS, and you referred to the elevated calcitriol levels in BS. Does the literature contain data regarding elevation of calcitriol levels in patients receiving loop diuretics?

Dr. Kurtz: Furosemide increases $1,25(OH)_2D$ in a chick renal tubule suspension, possibly via prostaglandin E_2-mediated stimulation of 1α hydroxylase activity [62]. In addition, chronic furosemide administration increases serum PTH, in contrast to thiazides, which decrease PTH levels [144–146]. I am unaware of any in-vivo data demonstrating an effect of furosemide on $1,25(OH)_2$ vitamin D. Your question addresses the more general issue as to whether the effects of loop diuretics mirror all the electrolyte and metabolic abnormalities of type-1 BS (NKCC2 mutations). One important and as-yet-unresolved finding is the absence of hypomagnesemia in patients with NKCC2 mutations despite the known inhibitory effect of loop diuretics on magnesium transport. It is perhaps significant that magnesium absorption in the thick ascending limb of Henle's loop is confined to the cortical portion [147]. Net magnesium transport in the medullary portion is essentially zero. Given the known distribution of NKCC2 isoforms that I discussed, if only those isoforms expressed in the medullary thick limb (NKCC2F and NKCC2AF) were mutated in type-1 BS, one might not expect to find abnormal loop magnesium transport, because cortical thick ascending limb function would remain unaffected. However, the NKCC2 mutations described thus far would be expected to affect the cortical and medullary thick ascending limb, as do loop diuretics. Therefore, isoform differences cannot explain the lack of hypomagnesemia in type-1 BS. In type-2 and type-3 BS, hypomagnesemia would also be expected.

In GS, the converse question arises. Thiazide diuretics do not characteristically cause more hypomagnesemia than do loop diuretics [148]. Therefore why is hypomagnesemia universally present in GS despite the lack of significant magnesium absorption in the distal convoluted tubule and collecting duct? One unproven explanation for the severe hypomagnesemia in GS is that defective sodium transport in the early distal convoluted tubule enhances sodium absorption more distally, resulting in an inhibition of putative basolateral Na^+/Mg^+ exchange. It is unclear why thiazide diuretics don't cause more severe hypomagnesemia; the same inhibition of apical Na^+/Mg^{2+} exchange should occur. Future studies in knockout mice will address the one or more mechanisms for the lack of hypomagnesemia in BS and the presence of hypomagnesemia in GS. This is one of several unresolved issues that will be the focus of future investigations: (*1*) What are the mechanisms for normal serum magnesium in BS and hypomagnesemia in GS? (*2*) Why is $1,25(OH)_2$ vitamin D elevated in BS? (*3*) Can patients with NKCC2, ROMK, and ClC-Kb mutations be clinically distinguished? (*4*) Why do patients with type-3 BS not have nephrocalcinosis? (*5*) Are other transporters with loss-of-function mutations involved in BS (KCC1)? (*6*) Is GS with chondrocalcinosis also due to mutations in the SLC12A3 gene locus? (*7*) What are the mechanisms for differences in clinical manifestations among patients who are members of the same family?

Dr. Kleeman: Has active transport of magnesium in the thick ascending limb of Henle been reported?

Dr. Kurtz: Studies of mouse and rabbit cortical thick ascending limb have shown that magnesium transport is a passive process driven by the lumen-positive potential difference [147]. Hormonal stimulation of passive magnesium absorption results from increases in transepithelial voltage and paracellular permeability. Active magnesium transport has not been reported in the TAL.

Dr. Madias: I have seen a few patients who, after gentamicin administration, have persistent hypokalemia, hypomagnesemia and magnesuria, chloride-resistant metabolic alkalosis, and high renin and aldosterone levels with normal renal function. Has anyone else here encountered similar patients?

Dr. Kurtz: Many years ago, Keating et al reported magnesium wasting in approximately 5% of patients treated with aminoglycosides [149]. The transport abnormality abated weeks to months after the drug was discontinued. Histologically, the proximal tubule is affected, as in *cis*-platinum toxicity. However, an isolated proximal tubule defect would not be expected to result in severe magnesium wasting. It is possible that aminoglycosides are inhibiting TAL transport by their ability to bind to the basolateral Ca^{2+}-sensing receptor (CaR) [150].

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REFERENCES

1. Stein JH: Nephrology Forum: The pathogenetic spectrum of Bartter's syndrome. *Kidney Int* 28:85–93, 1985
2. Gitelman HJ, Graham JB, Welt LG: A new familial disorder characterized by hypokalemia and hypomagnesemia. *Trans Assoc Am Phys* 79:221–235,
3. Gitelman HJ, Graham JB, Welt LG: A familial disorder characterized by hypokalemia and hypomagnesemia. *Ann NY Acad Sci* 162:856–864, 1969
4. Bartter FC, Pronove P, Gill JRJ, Macardle RC: Hyperplasia of the juxtaglomerular complex with hyperaldosteronism and hypokalemic alkalosis. *Am J Med* 33:811–828, 1962
5. Rosenbaum P, Hughes M: Persistent, probably congenital hypokalemic metabolic alkalosis with hyaline degeneration of the renal tubules and normal urinary aldosterone. *Am J Dis Child* 94:560, 1957
6. McCredie DA, Rotenberg E, Williams AL: Hypercalciuria in potassium-losing nephropathy: a variant of Bartter's syndrome. *Aust Pediatr J* 10:286–295, 1974
7. Gladziwa U, Schwarz R, Gitter AH, Bijman J, Seyberth H, Beck F, Ritz E, Gross P: Chronic hypokalaemia of adults: Gitelman's syndrome is frequent but classical Bartter's syndrome is rare. *Nephrol Dial Transplant* 10:1607–1613, 1995
8. Houser M, Zimmerman B, Davidman M, Smith C, Sinaiko A, Fish A: Idiopathic hypercalciuria associated with hyperreninemia and high urinary prostaglandin E. *Kidney Int* 26:176–182, 1984
9. Uribarri J, Alveranga D, Oh MS, Kukar NM, Del Monte ML, Carroll HJ: Bartter's syndrome due to a defect in salt reabsorption in the distal convoluted tubule. *Nephron* 40:52–56, 1985
10. Colussi G, Macaluso M, Brunati C, Minetti L: Calcium metabolism and calciotropic hormone levels in Gitelman's syndrome. *Miner Electrolyte Metab* 20:294–301, 1994
11. Rudin A, Sjogren B, Aurell M: Low urinary calcium excretion in Bartter's syndrome (*letter*). *N Engl J Med* 310:1190, 1984
12. Rudin A, Aurell M, Wilske J: Low urinary calcium excretion in Bartter's syndrome. *Scand J Urol Nephrol* 22:35–39, 1988
13. Sutton RA, Mavichak V, Halabe A, Wilkins GE: Bartter's syndrome: evidence suggesting a distal tubular defect in a hypocalciuric variant of the syndrome. *Miner Electrolyte Metab* 18:43–51, 1992
14. Bettinelli A, Bianchetti MG, Girardin E, et al: Use of calcium excretion values to distinguish two forms of primary renal tubular hypokalemic alkalosis: Bartter and Gitelman syndromes. *J Pediatr* 120:38–43, 1992
15. Mackie FE, Hodson EM, Roy LP, Knight JF: Neonatal Bartter syndrome–use of indomethacin in the newborn period and prevention of growth failure. *Pediatr Nephrol* 10:756–758, 1996
16. Proesmans W, Devlieger H, Van Assche A, Eggermont E, Vandenberghe K, Lemmens F, Sieprath P, Lijnen P: Bartter syndrome in two siblings–antenatal and neonatal observations. *Int J Pediatr Nephrol* 6:63–70, 1985
17. Seyberth HW: How can you differentiate neonatal Bartter's syndrome from hyperprostaglandin (-uria) E_2 syndrome? *Pediatr Nephrol* 8:407, 1994
18. Williams MP, Jones CL, Johnstone LM, Walker RG, McCredie DA, Powell HR: An extreme example of the neonatal form of Bartter's syndrome. *Pediatr Nephrol* 10:496–497, 1996
19. Wong W, Hulton SA, Taylor CM, Raafat F, Lote CJ, Lindop G: A case of neonatal Bartter's syndrome. *Pediatr Nephrol* 10:414–418, 1996
20. Proesmans WC: The neonatal form of Bartter's syndrome: current findings in etiology and physiopathology. *Verh K Acad Geneeskd Belg* 54:253–293, 1992
21. Deschenes G, Burguet A, Guyot C, Hubert P, Garabedian M, Dechaux M, Loirat C, Broyer M: Antenatal form of Bartter's syndrome. *Ann Pediatr* 40:95–101, 1993
22. Sieck UV, Ohlsson A: Fetal polyuria and hydramnios associated with Bartter's syndrome. *Obstet Gynecol* 63:22S–24S, 1984
23. James T, Holland NH, Preston D: Bartter syndrome. Typical facies and normal plasma volume. *Am J Dis Child* 129:1205–1207, 1975
24. Ohlson A, Sieck U, Cumming W, Akhtar M, Serenius F: A variant of Bartter's syndrome. *Acta Pediatr Scand* 73:868–874, 1984
25. Landau D, Shalev H, Ohaly M, Carmi R: Infantile variant of Bartter syndrome and sensorineural deafness: a new autosomal recessive disorder. *Am J Med Genet* 59:454–459, 1995
26. Massa G, Proesmans W, Devlieger H, Vandenberghe K, Van Assche A, Eggermont E: Electrolyte composition of the amniotic fluid in Bartter syndrome. *Eur J Obstet Gynecol Reprod Biol* 24:335–340, 1987
27. Shalev H, Ohaly M, Meizner I, Carmi R: Prenatal diagnosis of Bartter syndrome. *Prenat Diagn* 14:996–998, 1994
28. Seyberth HW, Rascher W, Schweer H, Kuhl PG, Mehls O, Scharer K: Congenital hypokalemia with hypercalciuria in preterm infants: a hyperprostaglandinuric tubular syndrome different from Bartter syndrome. *J Pediatr* 107:694–701, 1985
29. Seyberth HW, Koniger SJ, Rascher W, Kuhl PG, Schweer H: Role of prostaglandins in hyperprostaglandin E syndrome and in selected renal tubular disorders. *Pediatr Nephrol* 1:491–497, 1987
30. Fichman MP, Telfer N, Zia P, Speckart P, Golub M, Rude R: Role of prostaglandins in the pathogenesis of Bartter's syndrome. *Am J Med* 60:785–797, 1976
31. Bartter FC, Gill JR Jr, Frolich JC, Bowden RE, Hollifield JW, Radfar N, Keiser HR, Oates JA, Seyberth H, Taylor AA: Prostaglandins are overproduced by the kidneys and mediate hyperreninemia in Bartter's syndrome. *Trans Assoc Am Physicians* 89:77–91, 1976
32. Norby L, Flamenbaum W, Lentz R, Ramwell P: Prostaglandins and aspirin therapy in Bartter's syndrome. *Lancet* 2:604–606, 1976
33. Dunn MJ: Nephrology Forum: Prostaglandins and Bartter's syndrome. *Kidney Int* 19:86–102, 1981
34. Seidel C, Reinalter S, Seyberth HW, Scharer K: Pre-pubertal growth in the hyperprostaglandin E syndrome. *Pediatr Nephrol* 9:723–728, 1995
35. Pomeranz A, Korzets Z, Dolfin Z, Eliakim A, Bernheim Wolach B: Acute renal failure in the neonate induced by the administration of indomethacin as a tocolytic agent. *Nephrol Dial Transplant* 11:1139–1141, 1996
36. Cabrol D, Jannet D, Pannier E: Treatment of symptomatic polyhydramnios with indomethacin. *Eur J Obstet Gynecol* 66:11–15, 1996
37. McCredie DA: Variants of Bartter's syndrome. *Pediatr Nephrol* 10:419–421, 1996
38. Gitelman HG: Hypokalemia, hypomagnesemia, and alkalosis: a rose is a rose–or is it? *J Pediatr* 120:79–80, 1992
39. McCredie DA: Distinguishing between Bartter's syndrome and Gitelman's syndrome. *Pediatr Nephrol* 9:402, 1995
40. Bauer FM, Glasson P, Vallotton MB, Courvoisier B: Bartter's syndrome, chondrocalcinosis and hypomagnesemia. *Schweiz Med Wochenschr* 109:1251–1256, 1979
41. Resnick D, Rausch JM: Hypomagnesemia with chondrocalcinosis. *J Can Assoc Radiologists* 35:214–216, 1984
42. Salvarani C, Rossi F, Macchioni PL, Barrichi R, Cappozoli N, Castellani S, Ghirrelli L, Veneziani M, Scarti L, Portioli I: Bartter's syndrome and chondrocalcinosis: a possible role for hypomagnesemia in the deposition of calcium pyrophosphate dihydrate (CPPD) crystals. *Clin Exp Rheumatol* 7:415–420, 1989
43. de Heide LJ, Birkenhager JC: Bartter's syndrome, hypomagnesaemia and chondrocalcinosis. *Neth J Med* 39:148–152, 1991
44. Smilde TJ, Haverman JF, Schipper P, Hermus ARMM, van Leibergen FJHM, Jansen JLJ, Kloppenborg PWC, Koolen MI: Familial hypokalemia/hypomagnesemia and chondrocalcinosis. *J Rheumatol* 21:1515–1519, 1994
45. Fanconi A, Schachenmann G, Nussli RAP: Chronic hypokalemia with growth retardation, normotensive hyperrenin-hyperaldosteronism ("Bartter's syndrome") and hypercalciuria. *Helv Paediatr Acta* 26:144–163, 1971
46. Delaney VB, Oliver JF, Simms M, Costello J, Bourke E:

Bartter's syndrome: physiological and pharmacological studies. *Q J Med* 50:213–232, 1981
47. Restrepo de Rovetto C, Welch TR, Hug G, Clark KE, Bergstrom W: Hypercalciuria with Bartter syndrome: evidence for an abnormality of vitamin D metabolism. *J Pediatr* 115:397–404, 1989
48. Gill JR Jr, Frolich JC, Bowden RE, Taylor AA, Keiser HR, Seyberth WH, Oates JA, Bartter FC: Bartter's syndrome: a disorder characterized by high urinary prostaglandins and a dependence of hyperreninemia on prostaglandin synthesis. *Am J Med* 61:43–51, 1976
49. Yamada M, Matsumoto T, Takahashi N, Suda T, Ogata E: Stimulatory effect of prostaglandin E_2 on 1-alpha,25-dihydroxyvitamin D_3 synthesis in rats. *Biochem J* 216:237–240, 1983
50. Wark JD, Taft JL, Michelangeli VP, Veroni MC, Larkins RG: Biphasic action of prostaglandin E_2 on conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 in chick renal tubules. *Prostaglandins* 27:453–463, 1984
51. Kurose H, Sonn YM, Jafari A, Birge SJ, Avioli LV: Effects of prostaglandin E_2 and indomethacin on 25-hydroxyvitamin D_3-1-alpha-hydroxylase activity in isolated kidney cells of normal and streptozotocin-induced diabetic rats. *Calcif Tissue Int* 37:625–1985
52. Brouhard BH: Prostaglandins and hypokalemia. *J Pediatr* 107:738–740, 1985
53. Calo L, Cantaro S, Piccoli A, Favaro S, Bonfante L, Borsatti A: Full pattern of urinary prostaglandins in Bartter's syndrome. *Nephron* 56:451–452, 1990
54. Chan JC, Gill JR Jr, Bartter FC: Lack of evidence for a role for prostaglandins in the mediation of impaired urinary concentrating ability in Bartter's syndrome. *Nephron* 35:116–119, 1983
55. Hornych A, Krief C, Aumont J: Urinary prostaglandins in Bartter's and pseudo-Bartter's syndrome. *Uremia Invest* 9:203–210, 1985
56. Winterborn MH, Hewitt GJ, Mitchell MD: The role of prostaglandins in Bartter's syndrome. *Int J Pediatr Nephrol* 5:31–38, 1984
57. Lüthy C, Bettinelli A, Iselin S, Metta MG, Basilico E, Oetliker OH, Bianchetti MG: Normal prostaglandinuria E_2 in Gitelman's syndrome, the hypocalciuric variant of Bartter's syndrome. *Am J Kidney Dis* 25:824–828, 1995
58. Barbour GL, Day JO: Asymptomatic Bartter's syndrome. *South Med J* 71:1341–1344, 1978
59. Zipser RD, Rude RK, Zia PK, Fichman MP: Regulation of urinary prostaglandins in Bartter's syndrome. *Am J Med* 67:263–267, 1979
60. Dillon MJ, Shah V, Mitchell MD: Bartter's syndrome: 10 cases in childhood. Results of long-term indomethacin therapy. *Q J Med* 191:429–446, 1979
61. Carmine Z, Ettore B, Giuseppe C, Quirino M: The renal tubular defect of Bartter's syndrome. *Nephron* 32:140–148, 1982
62. Wark JD, Larkins RG, Eisman JA, Wilson KR: Regulation of 25-hydroxyvitamin D-1-alpha-hydroxylase in chick isolated renal tubules: effects of prostaglandin E_2, frusemide and acetylsalicylic acid. *Clin Sci* 61:53–59, 1981
63. Katayama S, Attallah AA, Stahl RA, Bloch DL, Lee JB: Mechanism of furosemide-induced natriuresis by direct stimulation of renal prostaglandin E_2. *Am J Physiol* 247:F555–F561, 1984
64. Zusman RM, Snider JJ, Cline A, Caldwell BV, Speroff L: Antihypertensive function of renal-cell carcinoma. Evidence for a prostaglandin-A-secreting tumor. *N Engl J Med* 290:843–845, 1974
65. Gill JR, Bartter FC: Evidence of prostaglandin-independent defect in chloride reabsorption in the loop of Henle as a proximal cause of Bartter's syndrome. *Am J Med* 65:766–772, 1978
66. Gill JR Jr: The role of chloride transport in the thick ascending limb in the pathogenesis of Bartter's syndrome. *Klin Wochenschr* 60:1212–1214, 1982
67. Puschett JB, Greenberg A, Mitro R, Piraino B, Wallia R: Variant of Bartter's syndrome with a distal tubular rather than loop of Henle defect. *Nephron* 50:205–211, 1988
68. Zarraga Larrondo S, Vallo A, Gainza J, Muniz R, Garcia Erauzkin G, Lampreabe I: Familial hypokalemia-hypomagnesemia or Gitelman's syndrome: a further case. *Nephron* 62:340–344, 1992
69. Tsukamoto T, Kobayashi T, Kawamoto K, Fukase M, Chihara K: Possible discrimination of Gitelman's syndrome from Bartter's syndrome by renal clearance study: report of two cases. *Am J Kidney Dis* 25:637–641, 1995
70. Kockerling A, Reinalter SC, Seyberth HW: Impaired response to furosemide in hyperprostaglandin E syndrome: evidence for a tubular defect in the loop of Henle. *J Pediatr* 129:519–528, 1996
71. Peters N, Bettinelli A, Spicher I, Basilico E, Metta MG, Bianchetti MG: Renal tubular function in children and adolescents with Gitelman's syndrome, the hypocalciuric variant of Bartter's syndrome. *Nephrol Dial Transplant* 10:1313–1319, 1995
72. Stokes JB: Electroneutral NaCl transport in the distal tubule. *Kidney Int* 36:427–433, 1989
73. Gesek FA, Friedman PA: On the mechanism of parathyroid hormone stimulation of calcium uptake by mouse distal convoluted tubule cells. *J Clin Invest* 90:749–758, 1992
74. Gamba G, Saltzberg SN, Lombardi M, Miyanoshita A, Lytton J, Hediger MA, Brenner BM, Hebert SC: Primary structure and functional expression of a cDNA encoding the thiazide-sensitive, electroneutral sodium-chloride cotransporter. *Proc Natl Acad Sci USA* 90:2749–2753, 1993
75. Gamba G, Miyanoshita A, Lombardi M, Lytton J, Lee WS, Hediger MA, Hebert SC: Molecular cloning, primary structure, and characterization of two members of the mammalian electroneutral sodium-(potassium)-chloride cotransporter family expressed in kidney. *J Biol Chem* 269:17713–17722, 1994
76. Bachmann S, Velazquez H, Reilly R, Moser D, Ellison D: Expression of the thiazide-sensitive cotransporter by rabbit distal convoluted tubule cells. *J Clin Invest* 96:2510–2514, 1995
77. Obermuller N, Bernstein P, Velasquez H, Reilly R, Moser D, Ellison DH, Bachmann S: Expression of the thiazide-sensitive Na-Cl cotransporter in rat and human kidney. *Am J Physiol* 269: F900–F910, 1995
78. Plotkin MD, Kaplan MR, Verlander JW, Lee WS, Brown D, Poch E, Guillans SR, Hebert SC: Localization of the thiazide-sensitive Na-Cl cotransporter, rTSC1, in the rat kidney. *Kidney Int* 50:174–183, 1996
79. Barry EL, Gesek FA, Kaplan MR, Hebert SC, Friedman PA: Expression of the sodium-chloride cotransporter in osteoblast-like cells: effect of thiazide diuretics. *Am J Physiol* 272:C109–C116, 1997
80. Abuladze N, Yanagawa N, Lee I, Jo OD, Newman D, Hwang J, Uyemura K, Pushkin A, Modlin RL, Kurtz I: Peripheral blood mononuclear cells express mutated NCCT mRNA in Gitelman's syndrome: evidence for abnormal NaCl cotransport. *J Am Soc Nephrol* 9:819–826, 1998
81. Lamberg B-A, Kuhlback B: Effect of chlorothiazide and hydrochlorothiazide on the excretion of calcium in the urine. *Scand J Lab Invest* 11:351–371, 1959
82. Costanzo LS: Mechanism of action of thiazide diuretics. *Semin Nephrol* 8:234–241, 1988
83. Gesek FA, Friedman PA: Mechanism of calcium transport stimulated by chlorothiazide in mouse distal convoluted tubule cells. *J Clin Invest* 90:429–438, 1992
84. White KE, Gesek FA, Freidman PA: Structural and functional analysis of Na^+/Ca^{2+} exchange in distal convoluted tubule cells. *Am J Physiol* 271:F560–F570, 1996
85. Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, Vaara I, Iwata F, Cushner HM, Koolen M, Gainza FJ, Gitelman JH, Lifton RP: Gitelman's variant of Bartter's syndrome; inherited hypokalaemia is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. *Nat Genet* 12:24–30, 1996
86. Simon DB, Karet FE, Hamden JM, Dipietro A, Sanjad SA, Lifton RP: Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na-K-Cl cotransporter NKCC2. *Nat Genet* 13:183–188, 1996
87. Simon DB, Karet FE, Rodriguez-Soriano J, Hamdan JH, Dipietro A, Trachtman H, Sanjad SA, Lifton RP: Genetic heterogeneity of Bartter's syndrome revealed by mutations in the channel, ROMK. *Nat Genet* 14:152–156, 1996
88. Pollak MR, Delaney VB, Graham RM, Hebert SC: Gitelman's syndrome (Bartter's variant) maps to the thiazide-sensitive cotransporter gene locus on chromosome 16q13 in a large kindred. *J Am Soc Nephrol* 7:2244–2248, 1996
89. Karolyi L, Ziegler A, Pollak M, Fischbach M, Grzeschik KH, Koch MC, Seyberth HW: Gitelman's syndrome is genetically distinct from other forms of Bartter's syndrome. *Pediatr Nephrol* 10:551–554, 1996
90. Lemmink HH, van den Heuvel LP, van Dijk HA, Merkx GF,

SMILDE TJ, TASCHNER PE, MONNENS LA, HEBERT SC, KNOERS NV: Linkage of Gitelman syndrome to the thiazide-sensitive sodium-chloride cotransporter gene with identification of mutations in Dutch families. *Pediatr Nephrol* 10:403–407, 1996
91. CHANG H, TASHIRO K, HIRAI M, IKEDA K, KUROKAWA K, FUJITA T: Identification of a cDNA encoding a thiazide-sensitive sodium-chloride cotransporter from the human and its mRNA expression in various tissues. *Biochem Biophys Res Commun* 223:324–328, 1996
92. MASTROIANNI N, BETTINELLI A, BIANCHETTI M, COLUSSI G, DE FUSCO M, SERENI F, BALLABIO A, CASARI G: Novel molecular variants of the Na-Cl cotransporter gene are responsible for Gitelman syndrome. *Am J Hum Genet* 59:1019–1026, 1996
93. RUDIN A: Bartter's syndrome. A review of 28 patients followed for 10 years. *Acta Medica Scand* 224:165–171, 1988
94. MUNOZ-FERNANDEZ S, PANTOJA L, MARTIN MOLA E, DE MIGUEL E, GIJON BANOS J: Chondrocalcinosis associated with Bartter's syndrome and hypomagnesemia. *J Rheumatol* 21:1782–1783, 1994
95. CHENG PT, PRITZKER KP, ADAMS ME, NYBURG SC, OMAR SA: Calcium pyrophosphate crystal formation in aqueous solutions. *J Rheumatol* 7:609–616, 1980
96. HUGHES AE, MCGIBBON D, WOODWARD E, DIXEY J, DOHERTY Localisation of a gene for chondrocalcinosis to chromosome 5p. *Hum Mol Genet* 4:1225–1228, 1995
97. ROCHA AS, KOKKO JP: Sodium chloride and water transport in the medullary thick ascending limb of Henle. *J Clin Invest* 52:612–623, 1973
98. BURG MB, GREEN N: Function of the thick ascending limb of Henle's loop. *Am J Physiol* 224:659–668, 1973
99. MOLONY DA, REEVES B, ANDREOLI TE: Na^+:K^+:$2Cl^-$ cotransport and the thick ascending limb. *Kidney Int* 36:418–426, 1989
100. KAPLAN MR, MOUNT DB, DELPIRE E, GAMBA G, HEBERT SC: Molecular mechanisms of NaCl cotransport. *Annu Rev Physiol* 58:649–668, 1996
101. PAYNE JA, FORBUSH B III: Alternatively spliced isoforms of the putative renal Na-K-Cl cotransporter are differentially distributed within the rabbit kidney. *Proc Natl Acad Sci USA* 91:4544–4548, 1994
102. YANG T, HUANG YG, SINGH I, SCHNERMANN J, BRIGGS JP: Localization of bumetanide- and thiazide-sensitive Na-K-Cl cotransporters along the rat nephron. *Am J Physiol* 271:F931–F939, 1996
103. KAPLAN MR, PLOTKIN MD, LEE W-S, XU Z-C, LYTTON J, HEBERT SC: Apical localization of the Na-K-Cl cotransporter, rBSCl, on rat thick ascending limbs. *Kidney Int* 49:40–47, 1996
104. XU JC, LYTLE C, ZHU TT, PAYNE JA, BENZ E, FORBUSH B III: Molecular cloning and functional expression of the bumetanide-sensitive Na-K-Cl cotransporter. *Proc Natl Acad Sci USA* 91:2201–2205, 1994
105. PAYNE JA, XU JC, HAAS M, LYTLE CY, WARD D, FORBUSH B III: Primary structure, functional expression, and chromosomal localization of the bumetanide-sensitive Na-K-Cl cotransporter in human colon. *J Biol Chem* 270:17977–17985, 1995
106. IGARASHI P, VANDEN HEUVEL GB, PAYNE JA, FORBUSH B III: Cloning, embryonic expression, and alternative splicing of a murine kidney-specific Na-K-Cl cotransporter. *Am J Physiol* 269:F405–F418, 1995
107. GILLEN CM, BRILL SB, PAYNE JA, FORBUSH B III: Molecular cloning and functional expression of the K-Cl cotransporter from rabbit, rat, and human. *J Biol Chem* 271:16237–16244, 1996
108. SASAKI S, ISHIBASHI K, YOSHIYAMA N, SHIGAI T: KCl co-transport across the basolateral membrane of rabbit renal proximal straight tubules. *J Clin Invest* 81:194–199, 1988
109. GREGER R, SCHLATTER E: Properties of the basolateral membrane of the cortical thick ascending limb of Henle's loop of rabbit kidney. *Pflügers Arch* 396:325–334, 1983
110. WANG W, HEBERT SC, GIEBISCH G: Renal K^+ channels: structure and function. *Annu Rev Physiol* 59:413–436, 1997
111. HO K, NICHOLS CG, LEDERER WJ, LYTTON J, VASSILEV PM, KANAZIRSKA MV, HEBERT SC: Cloning and expression of an inward rectifying ATP-regulated potassium channel. *Nature* 362:31–38, 1993
112. KONDO C, ISOMOTO S, MATSUMOTO S, YAMADA M, HORIO Y, YAMASHITA S, TAKEMURA-KAMEDA K, MATSUZAWA Y, KURACHI Y: Cloning and functional expression of a novel isoform of ROMK inwardly rectifying ATP-dependent K^+ channel, ROMK6 (Kirl.lf). *FEBS Lett* 399:122–126, 1996
113. SHUCK ME, BOCK JH, BENJAMIN CW, TSAI TD, LEE KS, SLIGHTON JL, BIENKOWSKI MJ: Cloning and characterization of multiple forms of the human kidney ROM-K potassium channel. *J Biol Chem* 269:24261–24270, 1994
114. HEBERT SC: An ATP-regulated, inwardly rectifying potassium channel from rat kidney (ROMK). *Kidney Int* 48:1010–1016, 1995
115. LEE WS, HEBERT SC: ROMK inwardly rectifying ATP-sensitive K^+ channel. I. Expression in rat distal nephron segments. *Am J Physiol* 268:F1124–F1131, 1995
116. BOIM MA, HO K, SCHUCK ME, BIENKOWSKI MJ, BLOCK JH, SLIGHTON JL, YANG Y, BRENNER BM, HEBERT SC: ROMK inwardly rectifying ATP-sensitive K^+ channel. II. Cloning and distribution of alternative forms. *Am J Physiol* 268:F1132–F1140, 1995
117. DERST C, KONRAD M, KOCKERLING A, KAROLYI L, DESCHENES G, DAUT J, KARSCHIN A, SEYBERTH HW: Mutations in the ROMK gene in antenatal Bartter syndrome are associated with impaired K^+ channel function. *Biochem Biophys Res Commun* 230:641–645, 1997
118. MCNICHOLAS CM, GUGGINO WB, SCHWIEBERT EM, HEBERT SC, GIEBISCH G, EGAN ME: Sensitivity of a renal K^+ channel (ROMK2) to the inhibitory sulfonylurea compound, glibenclamide, is enhanced by co-expression with the ATP-binding cassette transporter CFTR. *Proc Natl Acad Sci USA* 93:8083–8088, 1996
119. REEVES WB, WINTERS CJ, ZIMNIAK L, ANDREOLI TE: Epithelial chloride channels, from kidney to airway cells. *Adv Nephrol* 23:177–190, 1994
120. JENTSCH TJ, GÜNTHER W: Chloride channels: an emerging molecular picture. *Bioessays* 19:117–126, 1997
121. JENTSCH TJ, STEINMEYER K, SCHWARZ G: Primary structure of *Torpedo marmorata* chloride channel isolated by expression cloning in *Xenopus* oocytes. *Nature* 348:510–514, 1990
122. UCHIDA S, SASAKI S, FURUKAWA T, HIROAOKA M, IMAI T, HIRATA T, MARUMO F: Molecular cloning of a chloride channel that is regulated by dehydration and expressed predominantly in kidney medulla. *J Biol Chem* 268:3821–3824, 1993
123. KIEFERLE S, FONG P, BENS M, VANDEWALLE A, JENTSCH TJ: Two highly homologous members of the CLC chloride channel family in both rat and human kidney. *Proc Natl Acad Sci USA* 91:6943–6947, 1994
124. TAKEUCHI Y, UCHIDA S, MARUMO F, SASAKI S: Cloning, tissue distribution, and intrarenal localization of CLC chloride channels in human kidney. *Kidney Int* 48:1497–1503, 1995
125. VANDEWALLE A, CLUZEAUD F, BENS M, KIEFERLE S, STEINMEYER K, JENTSCH TJ: Localization and induction by dehydration of C1C-K chloride channels in the rat kidney. *Am J Physiol* 272:F678–F688, 1997
126. INTERNATIONAL COLLABORATIVE STUDY GROUP: Mutations in the gene encoding the inwardly-rectifying renal potassium channel, ROMK, cause the antenatal variant of Bartter's syndrome: evidence for genetic heterogeneity. International Collaborative Study Group for Bartter-like Syndromes. *Hum Mol Genet* 6:17–26, 1997
127. WANG T, WANG W-H, KLEIN-ROBBENHAAR G, GIEBISCH G: Effects of a novel K_{ATP} channel blocker on renal tubule function and K channel activity. *J Pharmacol Exp Ther* 273:1382–1389, 1995
128. SIMON DB, BINDRA RS, MANSFIELD TA, NELSON-WILLIAM C, MENDONCA E, STONE R, SCHURMAN S, NAYIR A, ALPAY H, BAKKALOGLU A, RODRIGUEZ-SORIANO J, MORALES JM, SANJAD SA, TAYLOR CM, PILZ D, BREM A, TRACHTMAN, GRISWOLD W, RICHARD GA, JOHN E, LIFTON RP: Mutations in the chloride channel gene, CLCNKB, cause Bartter's syndrome type III. *Nat Genet* 17:171–178, 1997
129. LLOYD SE, PEARCE SH, FISHER SE, STEINMEYER K, SCHWAPPACH B, SCHEINMAN SJ, HARDING B, BOLINO A, DEVOTO M, GOODYER P, RIGDEN SP, WRONG O, JENTSCH TJ, CRAIG IW, THAKKER RV: A common basis for three inherited kidney stone diseases. *Nature* 379:445–449, 1996
130. RUNEBERG L, COLLAN Y, JOKINEN EJ, LAHDEVIRTA J, ARO A: Hypomagnesemia due to renal disease of unknown etiology. *Am J Med* 59:873–881, 1975
131. GÜLLNER H-G, GILL JR JR, BARTTER FC, CHAN JCM, DICKMAN PS: A familial disorder with hypokalemic alkalosis, hyperreninemia, aldosteronism, high urinary prostaglandins and normal blood pressure that is not "Bartter's syndrome." *Trans Assoc Am Physicians* 90:175–188, 1979

132. GÜLLNER H-G, GILL JR JR, BARTTER FC: Correction of hypokalemia by magnesium repletion in familial hypokalemic alkalosis with tubulopathy. *Am J Med* 71:578–582, 1981
133. MEHROTRA R, NOLPH KD, KATHURIA P, DOTSON L: Hypokalemic metabolic alkalosis with hypomagnesuric hypermagnesemia and severe hypocalciuria: a new syndrome? *Am J Kidney Dis* 29:106–114, 1997
134. MORIOKA A, IWASHIRO M, MATSUBAYASHI Y, TERAMURA Y, KURIBAYASHI K: Effects of non-MHC background genes on the induction of CD4+ T cells that prevent rejection of a highly immunogenic tumor, FBl-3. *Int Immunol* 6:839–846, 1994
135. RATKAY LG, TAIT B, TONZETICH J, WATERFIELD JD: Lpr and MRL gene involvement in the control of adjuvant enhanced arthritis in MRL-lpr mice. *J Autoimmunol* 7:561–573, 1994
136. VUKUSIC B, POPLONSKI L, PHILLIPS L, PAULNG J, DELOVITCH T, HOZUMI N, WITHER J: Both MHC and background gene heterozygosity alter T cell receptor repertoire in an antigen-specific response. *Mol Immunol* 32:1355–1367, 1995
137. KUNCHAPARTY S, ALI N, BERNSTEIN PL, DESIR GV, ELLISON DH: A carboxyterminal mutation of mouse thiazide-sensitive Na-Cl cotransporter abolishes function (*abstract*). *J Am Soc Nephrol* 7:1284, 1996
138. KAJI DM, CHASE HS JR, ENG JP, DIAZ J: Prostaglandin E2 inhibits Na-K-2Cl cotransport in medullary thick ascending limb cells. *Am J Physiol* 271:C354–C361, 1996
139. MACICA CM, YANG Y, HEBERT SC, WANG WH: Arachidonic acid inhibits activity of cloned renal K^+ channel, ROMK1. *Am J Physiol* 271:F588–F594, 1996
140. OZCAN T, KALELI B, OZEREN M, TURAN C, ZORLU G: Urinary calcium to creatinine ratio for predicting preeclampsia. *Am J Perinatol* 12:349–351, 1995
141. TOLAYAMAT A, SANCHEZ-RAMOS L, YERGLEY AL, VIEIRA NE, ABRAMS SA, EDELSTEIN P: Pathophysiology of hypocalciuria in preeclampsia: measurement of intestinal calcium absorption. *Obstet Gynecol* 83:239–243, 1994
142. BOURGES H, HALHALI A: Hypothesis to explain the association between hypocalciuria and low circulating 1,25-dihydroxyvitamin D in preeclampsia. *Med Hypotheses* 41:239–243, 1993
143. SEALY JE, ITSKOVITZ-ELDOR J, RUBATTU S, JAMES JD, AUGUST P, THALER I, LEVRON J, LARAGH JH: Estradiol-and progesterone-related increases in the renin-aldosterone system: studies during ovarian stimulation and early pregnancy. *J Clin Endocrinol Metab* 79:259–264, 1994
144. STOTE RM, SMITH LH, WISON DM, DUBE WJ, GOLDSMITH RS, ARNAUD CD: Hydrochlorothiazide effects on serum calcium and immunoreactive parathyroid hormone concentrations. *Ann Intern Med* 77:587–591, 1972
145. COE FL, CANTERBURY JM, FIRPO JJ, REISS E: Evidence for secondary hyperparathyroidism in idiopathic hypercalciuria. *J Clin Invest* 52:134–142, 1973
146. STEIN MS, SCHERER SC, WALTON SL, GILBERT RE, EBELING PR, FLICKER L, WARK JD: Risk factors for secondary hyperparathyroidism in a nursing home population. *Clin Endocrinol* 44:375–383, 1996
147. DE ROUFFIGNAC C, MANDON B, WITTNER M, DI STEFANO A: Hormonal control of renal magnesium handling. *Miner Electrolyte Metab* 19:226–231, 1993
148. SUTTON RA, DOMRONGKITCHAIPORN S: Abnormal renal magnesium handling. *Miner Electrolyte Metab* 19:232–240, 1993
149. KEATING MJ, SETHI MR, BODEY GP, SAMAAN NA: Hypocalcemia with hypoparathyroidism and renal tubular dysfunction associated with aminoglycoside therapy. *Cancer* 39:1410–1414, 1977
150. WANG W, LU M, BELAZY M, HEBERT SC: Phospholipase A2 is involved in mediating the effect of extracellular Ca^{2+} on apical K^+ channels in the rat TAL. *Am J Physiol* 273:F421–F429, 1997