

# Inherited Renal Tubulopathies Associated With Metabolic Alkalosis: Effects on Blood Pressure

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Inherited tubular disorders associated with metabolic alkalosis are caused by several gene mutations encoding different tubular transporters responsible for NaCl renal handling. Body volume and renin-angiotensin-aldosterone system status are determined by NaCl reabsorption in the distal nephron. Two common hallmarks in affected individuals: hypokalemia and normal / high blood pressure, support the differential diagnosis. Bartter's syndrome, characterized by hypokalemia and normal blood pressure, is a heterogenic disease caused by the loss of function of SLC12A1 (type 1), KCNJ1 (type 2), CLCNKB (type 3), or BSND genes (type 4). As a result, patients present with renal salt wasting and hypercalciuria. Gitelman's syndrome is caused by the loss of function of the SLC12A3 gene and may resemble Bartter's syndrome, though is associated with the very low urinary calcium. Liddle's syndrome, also with similar phenotype but with hypertension, is produced by the gain of function of the SNCC1B or SNCC1G genes, and must be distinguished from other entities of inherited hypertension such as Apparently Mineralocorticoid Excess, of glucocorticoid remediable hypertension.

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Integrity of renal sodium chloride (NaCl) reabsorption is critical for body volume balance preservation.<sup>1</sup> The regulation of distal nephron NaCl handling accounts for the final amount of salt excreted by urine, and thus, involved tubular transport mechanisms are major contributors to maintain salt and water homeostasis.<sup>2</sup>

In the thick ascending limb of loop of Henle, NaCl reabsorption is maintained by 3 principal transport systems. Luminal NaCl enters the cell via the bumetanide-sensitive Na-2Cl-K cotransporter (NKCC2 or BSC1),<sup>3</sup> whereas potassium (K) is recycled into the lumen via an adenosine triphosphate (ATP)-sensitive K channel (ROMK), necessary for NKCC2 activity.<sup>4</sup> In addition, Cl<sup>-</sup> leaves the cell via the basolateral membrane through a Cl<sup>-</sup> channel composed of 2 units, or cotransported with K<sup>+</sup>; whereas Na<sup>+</sup> exits the cell through the Na/K-ATPase system. Recirculation of K<sup>+</sup> to the lumen and the exit of Cl<sup>-</sup>

across the basolateral membrane generate a lumen-positive transepithelial voltage gradient that drives paracellular Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> reabsorption.<sup>5</sup> In the early distal convoluted tubule, NaCl reabsorption is mediated by the luminal NaCl cotransporter<sup>6</sup> and leaves the cell through specific Cl<sup>-</sup> channels,<sup>7</sup> again composed of ClC-Kb and barttin,<sup>8</sup> and through the Na<sup>+</sup>/K<sup>+</sup>/ATPase channel as well. The same cells reabsorb calcium (Ca<sup>2+</sup>) by a specific luminal Ca<sup>2+</sup> channel and basolateral Na<sup>+</sup>/Ca<sup>2+</sup> exchange.<sup>5</sup> At the principal cells of the collecting duct, Na<sup>+</sup> is reabsorbed via the epithelial Na<sup>+</sup> channel (ENaC) and exits by the Na<sup>+</sup>/K<sup>+</sup>/ATPase.<sup>8</sup> Luminal Na<sup>+</sup> entry is accomplished with K<sup>+</sup> extrusion by ROMK. Aldosterone stimulates the mineralocorticoid receptor, and increases the ENaC, Na<sup>+</sup>/K<sup>+</sup>/ATPase, and ROMK channel activities.<sup>4</sup> Furthermore, mineralocorticoids increase the serum- and glucocorticoid-inducible kinase transcription, which also activates ENaC, Na<sup>+</sup>/K<sup>+</sup>/ATPase, and ROMK.<sup>9</sup> Glucocorticoids may interact with the mineralocorticoid receptor, although usually they are inactivated by 11 $\beta$ -hydroxysteroid dehydrogenase previously.<sup>10</sup>

Several gene mutations encoding different tubular transport systems responsible for NaCl handling in the distal nephron

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Table 1 Genetics of Inherited Disorders of Renal NaCl Transport

Inherited Disorder	Type of Inheritance	Gene Localization	Gene Symbol	Gene Product	Gene Function
Antenatal Bartter's syndrome (type 1)	AR	15q15-21	SLC12A1	NKCC2	Loss-of-function
Antenatal Bartter's syndrome (type 2)	AR	11q24	KCNJ1	ROMK	Loss-of-function
Classic Bartter's syndrome (type 3)	AR	1p36	CLCNKB	CIC-Kb	Loss-of-function
Antenatal Bartter's syndrome with neurosensorial deafness (type 4)	AR	1 p32.3	BSND	Barttin	Loss-of-function
Hypocalcemia with Bartter's syndrome (type 5)	AD	3q21.1	CaSR	CaSR	Gain-of-function
Antenatal Bartter's syndrome with neurosensorial deafness (type 6)	AR	1p36	CLCNKA/ CLCNKB	CIC-Ka/CIC-Kb	Loss-of-function
Gitelman's syndrome	AR	16q13	SLC12A3	NCC	Loss-of-function
Liddle's syndrome	AD	16p12	SNCC1B, SNCC1G	$\beta$ , $\gamma$ ENaC	Gain-of-function
AME	AR	16q12	HSD11B2	11- $\beta$ -HSD2	Loss-of-function
Familial hypertension secondary to activated mineralocorticoid receptor	AD	4q31.1	MR	MC receptor	Gain-of-function
GRA	AD	8q22	CYP11B1/ CYP11B2	11- $\beta$ -hydroxylase/aldose synthase	Chimeric gene

Abbreviations: AD, autosomal dominant; AR, autosomal recessive.

have been described (Table 1). Thus, some inherited disorders produce impaired Na<sup>+</sup> reabsorption, leading to volume depletion and secondary activation of the renin-angiotensin-aldosterone system (RAAS). Conversely, other monogenic diseases enhance tubular Na<sup>+</sup> retention and cause subsequent volume expansion and RAAS suppression.<sup>11,12</sup> Independently of the underlying defects, patients' phenotype is characterized by 2 hallmarks: low plasma K<sup>+</sup> levels caused by renal salt wasting, and metabolic alkalosis. Differential diagnosis is based on the blood pressure and RAAS status. Increasing interest in inherited tubulopathies affecting renal salt handling is justified by the need for better understanding of body volume regulation, identification of genetic protective or risk factors for hypertension, and their putative role on optimal blood pressure control in the general population as well.<sup>11,12</sup>

## Inherited Tubulopathies With Metabolic Alkalosis

Patients with inherited tubulopathies leading to metabolic alkalosis are clinically characterized by hypokalemia and increased K<sup>+</sup> in urine (>20 mEq/L).<sup>13</sup> Other nonrenal disorders impairing the tubular NaCl transport and diseases with body volume imbalance could mimic tubulopathies and

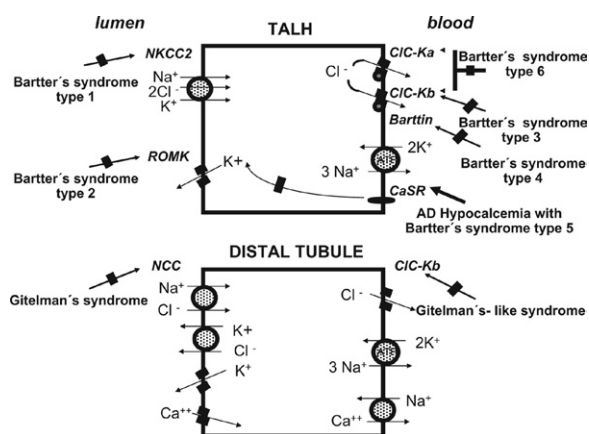
therefore need to be excluded before establishing a definitive diagnosis. Differential diagnosis should include causes of hypokalemia and volume contraction such as K<sup>+</sup>-deficient intake, or increased K<sup>+</sup> wasting of gastrointestinal<sup>14</sup> or skin origin,<sup>15</sup> all resulting in a low K<sup>+</sup> concentration in the urine (<20 mEq/L).

## Inherited Tubulopathies With Hypokalemia and Normotension: Bartter's and Bartter's-Like Syndromes

Bartter's syndrome and Bartter's-like syndromes produce renal salt wasting caused by the loss of function of specific tubular transport mechanisms involved in distal NaCl reabsorption (Fig 1).<sup>16</sup>

### Bartter's Syndrome

In 1962, Bartter et al<sup>17</sup> reported on 2 patients with a new syndrome characterized by hypokalemia, metabolic alkalosis, hyperaldosteronism with normal blood pressure, de-



**Figure 1** Disturbed renal transport systems in Bartter's and Gitelman's syndromes. TALH, thick ascending limb of loop of Henle.

creased pressor responsiveness to infused angiotensin II, and juxtaglomerular apparatus hyperplasia. Nowadays it is evident that Bartter's syndrome includes a variety of different disorders of distal NaCl renal handling and these are referred as *Bartter's-like syndromes*. Three different clinical entities can be distinguished: antenatal Bartter's syndrome, classic Bartter's syndrome, and Gitelman's syndrome.

## Antenatal Bartter's Syndrome

### Clinical and Biochemical Features

Antenatal Bartter's syndrome represents a severe variant of the disease.<sup>18,19</sup> Clinical signs start during pregnancy and include marked polyhydramnios as a result of fetal polyuria, premature delivery, and life-threatening episodes of polyuria, dehydration, hypercalciuria, and early onset nephrocalcinosis. Diuresis is as large as 12 to 50 mL/kg/h and persists during at least the first month of life. Some patients are phenotypic: skinny, with poor muscle mass, and triangular-shaped facies characterized by a prominent forehead, large eyes, protruding ears, and a drooping mouth. Strabismus and sensorineural deafness have been reported too. Failure to thrive and growth retardation are the rule, but appropriate therapy allows optimal growth. Systemic manifestations such as osteopenia, fever, secretory diarrhea, convulsions, and increased susceptibility to infection also have been observed.

### Genetics

Antenatal Bartter's syndrome is a heterogenic autosomal-recessive entity. In the less-frequent type 1, mostly described in consanguineous families, mutations in the SLC12A1 gene, located on 15q15 to 21, encoding the NKCC2 transporter in the TALH, cause impaired NaCl reabsorption.<sup>20</sup> Frameshift, nonsense, and missense mutations in the SLC12A1 gene, usually in homozygosis, have been described.<sup>21-25</sup> In some patients, the disease can start beyond the newborn period.<sup>25</sup> Functional studies have shown that the loss of function of the NKCC2 transporter is caused by reduced expression of the mutant protein and not by a trafficking defect.<sup>26</sup>

Individuals with type 2 antenatal Bartter's syndrome show frameshift, nonsense, and missense mutations in the ROMK

gene, sited on chromosome 11q24. Most mutations are located in exon 5, but not exclusively.<sup>27-31</sup> In contrast to SLC12A1 gene mutations, most of the investigated ROMK mutations displayed a trafficking defect that might be rescued by pharmacologic agents, such as aminoglycosides, acting as molecular chaperones.<sup>31</sup>

Recently, mutations in a third gene were found to produce a peculiar type of antenatal Bartter's syndrome, so-called *type 4*, associated with neurosensorial deafness and early chronic renal insufficiency.<sup>32</sup> This BSND gene is localized on 1p32.3, and encodes a novel protein named *barttin*.<sup>33</sup> ClC-K-barttin heteromers are critical for NaCl reabsorption and K recycling in the TAL and inner ear.<sup>6</sup> This  $\beta$ -subunit is required for the trafficking of the ClC-K (both ClC-Ka and ClC-Kb) to the plasma membrane. Expression studies of mutant barttins have shown its retention in intracellular organelles.<sup>34</sup>

In addition, some patients with Bartter's syndrome type 3, caused by mutations or deletions in the CLCNKB gene, occasionally may present polyhydramnios and symptoms during the neonatal period as well.<sup>35</sup> Combined impairment of chloride channel functions of ClC-Ka and ClC-Kb owing to associated mutations in both CLCNKA and CLCNKB genes (Bartter's syndrome type 6) also may mimic this phenotype.<sup>36</sup> Both ClC-Ka and ClC-Kb are associated with the  $\beta$ -subunit barttin and are expressed in the middle ear but differ in their distribution along the nephron: ClC-Ka predominates in the thin ascending loop of Henle whereas the expression of ClC-Kb extends from the thick ascending limb to the cortical collecting duct. Interestingly, the mouse *Clcnk1*<sup>-/-</sup> (equivalent to homozygous ClC-Ka mutations) shows nephrogenic diabetes insipidus without salt wasting.<sup>37</sup>

### Pathogenesis

Impaired ROMK function results in decreased K concentration in the TAL lumen, and defective NKCC2 activity, which is responsible for 30% of the total filtered NaCl. It results in sodium wasting, volume contraction, and RAAS stimulation.<sup>38,39</sup> Increased prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) secretion further aggravates the picture by both independent stimulation of the RAAS and inhibition of ROMK channel and NaCl transport in the thick ascending limb of loop of Henle.<sup>2</sup> Thus, patients with antenatal Bartter's syndrome show a blunted natriuretic and hormonal response to furosemide compared with controls.<sup>40</sup> Chronic blockade of the NKCC2 transporter by furosemide causes symptoms similar to those observed in antenatal Bartter's syndrome, thus providing a useful animal model. This effect of furosemide was blunted when administered to genetically modified mice with knocked-out EP1, EP3, and EP4 PGE<sub>2</sub> receptors, but not when administered to mice with knocked-out EP2 PGE<sub>2</sub> receptors or IP prostacyclin receptors.<sup>41</sup>

The characteristic presence of hypercalciuria is a consequence of transepithelial voltage gradient loss caused by defective Na-K-Cl reabsorption because about 25% of filtered Ca is reabsorbed in the TAL coupled to NKCC2 activity.<sup>5</sup> Hypercalciuria also has been related to an excessive synthesis of 1,25-dihydroxy-vitamin D, leading to increased bone resorption and to a renal leak of Ca.<sup>42</sup>

## Diagnosis

Antenatal Bartter's syndrome is very characteristic.<sup>18,19,22</sup> Prenatal diagnosis can be made based on the high  $\text{Cl}^-$  content in the amniotic fluid.<sup>43</sup> Remarkably,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{PGE}_2$  concentrations in the amniotic fluid are normal. A pregnant mother's urine also may be useful for prenatal diagnosis because it may show very low concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{Ca}^{2+}$ .<sup>44</sup> Mutational analysis of genomic DNA in amniocytes can establish the definitive prenatal diagnosis.<sup>45</sup>

## Differential Diagnosis

Newborns with type 2 ROMK mutations often present with mild  $\text{NaCl}$  wasting and may resemble a picture of primary pseudohypoaldosteronism type 1 with hyponatremia, hyperkalemia, and metabolic acidosis.<sup>46</sup> Beyond the neonatal period, renal potassium wasting, leading to hypokalemia and metabolic alkalosis, becomes very apparent, and the Bartter's symptoms turn typical. However, prematurity, hydramnios, and hypercalciuria, which are common in both diseases, may confuse the current diagnosis, mostly in the first days of life.

According to our personal observations, the syndrome of familial hypomagnesemia-hypercalciuria with nephrocalcinosis also can be similar to antenatal Bartter's syndrome because hypercalciuria and nephrocalcinosis already are present in the newborn, but significant hypomagnesemia may be absent.<sup>47</sup> Pseudoantenatal Bartter's syndrome has been reported in a preterm baby with complex cyanotic heart disease, receiving high doses of  $\text{PGE}_1$  to maintain the ductus arteriosus open.<sup>48</sup>

## Treatment and Prognosis

PG synthetase inhibitor, mainly indomethacin, is the drug of choice for treatment, but should be delayed until 4 to 6 weeks of life because of its side effects. After this time, indomethacin is very efficient for neutralizing the amplifying effect caused by PGs. Prenatal therapy with this medicine has been advocated, although extreme caution is advisable because of its potential risk in the fetus.<sup>49</sup> Young infants with ROMK mutations are very sensitive to indomethacin and require a low dose ( $<1$  mg/kg/d) to maintain normal kalemia without K supplements. Indeed, they could even present with hyperkalemia. Eventually, most patients require KCl supplements with time. In general, treated patients maintain an excellent clinical condition with optimal growth, and puberty and intellectual development also are normal.<sup>13</sup> Reversely, most but not all infants with BSND mutations develop progressive chronic renal failure as a result of indomethacin resistance.<sup>50,51</sup> After birth, aggressive management of dehydration and electrolytic imbalance is recommended. Despite indomethacin, hypercalciuria remains largely increased, a risk factor for progression of nephrocalcinosis. Other adjuvant therapies such as amiloride, hydrochlorothiazide, and potassium phosphate have been ineffective to control nephrocalcinosis.<sup>2</sup> Thus, some children reach end-stage chronic kidney disease as a result of chronic tubulointerstitial nephropathy.<sup>13</sup> Recently, specific cyclooxygenase-2 inhibition by nimesuline in neonatal Bartter's syndrome<sup>52</sup> has been observed to impressively improve hyperprostaglandinuria, secondary hyperaldoste-

ronism, and hypercalciuria,<sup>53,54</sup> but its use remains experimental.

## Classic Bartter's Syndrome

### Clinical and Biochemical Features

Bartter's typical picture is represented by an infant with polyuria, polydipsia, vomiting, constipation, salt craving, tendency to dehydration, and failure to thrive. Often symptoms begin soon after birth, but a history of hydramnios and premature delivery also is frequent.<sup>13</sup> In addition, fatigue, muscle weakness, cramps, and tetany episodes may be observed later in childhood. Some patients have a distinctive facies phenotype attributed to the effect of hypokalemia on facial muscles. Developmental delay, mild brain dysfunction, nephrocalcinosis, and polyuria-related ureterohydronephrosis have been reported too. In cases with severe and chronic hypokalemia, medullary cysts are described.<sup>2</sup>

Bartter's syndrome hallmark is the presence of hypokalemia between 1.5 and 2.5 mEq/L. Hypochloremia and metabolic alkalosis are almost constant, but occasionally a patient may suffer metabolic acidosis. Hyperuricemia due to volume contraction is found in half the patients, but hypomagnesemia is observed less frequently. In urine, there is salt-wasting and increased fractional excretion of  $\text{K}^+$ ,  $\text{Na}^+$ , and  $\text{Cl}^-$ , and normal or high urinary Ca excretion.<sup>55</sup> Defects in concentrating and diluting abilities are constant owing to both hypokalemia and impaired  $\text{NaCl}$  reabsorption in the TAL. The glomerular filtration rate is preserved in early stages of the disease but may decrease in untreated patients as a result of chronic hypokalemia or tubulointerstitial damage. Hyperreninemia is constant and usually is associated with increased aldosterone in plasma, although aldosterone secretion can be suppressed by  $\text{K}^+$  depletion. Paradoxically, blood pressure is normal owing to vascular hyporeactivity despite increased blood levels of renin, angiotensin II, norepinephrine, and endothelin.<sup>56</sup> This complex phenomenon is explained by vascular underfilling, increased PG, and possibly increased nitric oxide synthesis as well.<sup>57</sup> Remarkably, PG excretion in urine is high, mostly  $\text{PGE}_2$  and 6-keto- $\text{PGF}_{1\alpha}$ , the main metabolite of prostaglandin  $\text{I}_2$  or prostacyclin.<sup>58</sup>

### Genetics

The so-called classic form, or Bartter's syndrome type 3, is an autosomal-recessive entity caused by mutations in the *CLCNKB* gene, on 1p36 chromosome, encoding the CLC-Kb channel. At present, at least 26 large deletions or nonsense, missense, and splice mutations have been reported. Homozygous deletion encompassing a great part of the *CLCNKB* gene represents the most common molecular finding and has been reported in at least 30 patients with Bartter's syndrome.<sup>59-61</sup> This observation is unlikely to be the consequence of a founder effect given the widely varying ethnic origins of the patients. A founder mutation in the *CLCNKB* gene (A204T) causes Bartter's syndrome type III in Spain.<sup>62</sup> An exceptional family has been reported with 2 siblings presenting compound heterozygous mutations in the *CLCNKB* gene and in the *SLC12A3* gene encoding the thiazide-sensitive luminal  $\text{NaCl}$  cotransporter.<sup>63</sup>

In addition, severe gain-of-function mutations in the extracellular  $\text{Ca}^{2+}$ -sensing receptor can result in a Bartter-like syndrome because activation of this G-protein-coupled receptor, which is expressed highly in the basolateral membranes of the ascending limb of loop of Henle, inhibits salt transport through ROMK inhibition (Bartter syndrome type 5).<sup>64,65</sup> Delayed diagnosis in some patients with neonatal Bartter's syndrome caused by defects in NKCC2 or ROMK encoding genes may also resemble the classic form.<sup>23-25</sup>

A common polymorphism (T481S variant) in the ClC-Kb channel may be associated with essential hypertension. This substitution is present in 20% to 40% of the population and induces a 7-fold increase in Cl transport by this channel in the *Xenopus* oocytes.<sup>66</sup> If this finding is confirmed it would be an important step in understanding blood pressure regulation in healthy individuals.

### Pathogenesis

The ClC-Kb channel malfunction reduces the exit of  $\text{Cl}^-$  and modifies the Na-K-Cl reabsorption in the TAL by modifying the transepithelial voltage gradient.<sup>5</sup> Renal salt wasting is not as massive as in neonatal Bartter's syndrome because  $\text{Cl}^-$  leaves the cell through other channels, cotransported with  $\text{K}^+$ .<sup>5</sup> Impaired NaCl reabsorption at the TAL in classic Bartter's syndrome was long suspected based on fractional clearances of Cl during hypotonic saline diuresis. The normal index of distal  $\text{Cl}^-$  reabsorption ( $C_{\text{H}_2\text{O}}/C_{\text{H}_2\text{O}} + C_{\text{Cl}} \times 100$ ) greater than 80%,<sup>67</sup> is reduced in these patients often to less than 60%.<sup>68</sup>

### Differential Diagnosis

Primary Fanconi syndrome or cystinosis with hypokalemia and metabolic alkalosis could mimic Bartter's syndrome. Occasionally, children with Kearns-Sayre syndrome may resemble those with Bartter's syndrome.<sup>69</sup> Rarely, an entity named *familial hypokalemic alkalosis with proximal tubulopathy* (Güller's syndrome)<sup>70</sup> shares some characteristics with Bartter's syndrome too.<sup>70,71</sup>

Gitelman's syndrome is an inherited salt-wasting tubulopathy that has been confused with Bartter's syndrome for a long time. Several studies have shown that many individuals with Bartter's syndrome type 3 show a mixed Bartter-Gitelman phenotype, consistent with the role of this  $\text{Cl}^-$  channel in both the thick ascending loop of Henle and the distal collecting tubule.<sup>60,61</sup> Nowadays the genetic diagnosis provides evidence that both entities represent different diseases. A complete description of Gitelman's syndrome is provided later.

Other renal abnormality that mimic Bartter's syndrome and needed to be excluded is the surreptitious use of loop diuretics such as furosemide. Detection of diuretics in the urine may help to establish the diagnosis.<sup>72,73</sup>

### Treatment and Prognosis

Correction of hypokalemia constitutes the aim of treatment. Potassium supplements such as KCl (1-3 mEq/kg/d, or more) are always necessary, but inefficient as unique therapy because it is quickly lost in the urine. The addition of spironolactone (10-15 mg/kg/d) or triamterene (10 mg/kg/d) may be

useful, but their effect often is transient. PG synthetase inhibitors are the drugs of choice: indomethacin (2-5 mg/kg/d), acetylsalicylic acid (100 mg/kg/d), ibuprofen (30 mg/kg/d), or ketoprofen (20 mg/kg/d) produce a spectacular response with improved well-being, strength, and activity; diminution of polyuria and polydipsia; and reinstatement of normal, or even catch-up, growth. There is an immediate increase in plasma K levels, however, which rarely exceeds 3.5 mEq/L. Plasma renin activity and aldosterone levels decrease toward a normal range, and the vascular response to angiotensin II or noradrenalin also normalizes.<sup>2</sup> Indomethacin is the most frequent drug used and although it usually is well tolerated, attention should be paid to its digestive or bone marrow side effects. The addition of Mg salts always should be considered if hypomagnesemia is present because Mg deficiency can increase K wasting.<sup>13,14</sup> The long-term efficacy of treatment has been established clearly but, in adulthood, recurrences resembling Gitelman's syndrome phenotype are described. The long-term prognosis remains guarded, however, and the lack of rigorous therapeutic control may lead to slow progression to chronic renal failure.<sup>74</sup>

## Gitelman's Syndrome

### Clinical and Biochemical Features

In 1966, Gitelman et al<sup>75</sup> described 3 adults, 2 of them siblings, with transient muscle weakness and tetany episodes. They suffered chronic hypokalemia and hypomagnesemia because of the excessive wasting of K and Mg in the urine, in the absence of any other additional tubular disorder. Many contemporary reports in children and young adults<sup>47</sup> have confirmed that Gitelman's syndrome or familial hypokalemia-hypomagnesemia is an autosomal-recessive tubulopathy, clinically defined by recurrent tetany episodes, without polyuria or growth failure. Eventually, chondrocalcinosis<sup>76</sup> and sclerochoroidal calcification<sup>77</sup> also have been reported. Less frequently, patients show hypokalemia, hypomagnesemia, a normal glomerular filtration rate, and metabolic alkalosis associated with increased plasma renin, aldosterone, and prostaglandins levels. In addition to renal K and Mg loss, hypocalciuria constitutes the characteristic hallmark of the disease.<sup>55</sup> Some affected individuals may remain asymptomatic for long periods of time, with occasional weakness and tetany episodes associated with febrile episodes or vomits.<sup>47</sup> The disease-free intervals may be prolonged, and in many cases diagnosis is made only during adult life. However, almost half of the patients complain of salt craving; musculoskeletal symptoms such as cramps, muscle weakness, and aches; and constitutional symptoms such as fatigue, dizziness, nocturia, and polydipsia.<sup>78</sup>

### Genetics

Gitelman's syndrome is caused by mutations of the SLC12A3 gene, located on 16q13 chromosome, encoding the synthesis of the thiazide-sensitive luminal NaCl cotransporter (NCC) at the early distal convoluted tubule.<sup>79</sup> This mechanism explains the profound homology between affected individuals and those on chronic treatment with thiazides.<sup>80</sup> To date, more than 100 different mutations, including missense, non-

sense, frameshift, deletion, insertion, and splice-site mutations have been identified in patients with Gitelman's syndrome. The mutations are located throughout the coding sequence of the gene but most of these mutations are found in the intracellular domains of the protein.<sup>81,82</sup> A specific mutation (intron 9 + 1G>T) is linked to Gitelman's syndrome in Gypsies.<sup>83</sup> There is a marked phenotypic variability that may depend partly on gender differences because the degree of hypokalemia is more profound in males than in females.<sup>84</sup>

Mutant proteins identified in Gitelman's syndrome are either improperly glycosylated proteins that are retained in the endoplasmic reticulum and/or pre-Golgi complex thus failing to reach the cell surface (class I), or functional mutants that normally are glycosylated but that are partly impaired in their routing to the cell membrane (class II).<sup>85</sup>

### Pathogenesis

SLC12A3-mutated alleles produce impaired NaCl reabsorption at the distal tubule, leading to volume contraction and RAAS activation. All of these increase distal Na<sup>+</sup> reabsorption through the ENaC and K<sup>+</sup> secretion, explaining the characteristic hypokalemia. Further, subsequent stimulation of K<sup>+</sup> reabsorption mechanisms (H<sup>+</sup>/K<sup>+</sup> ATPase) in the intercalated cells of the medullar collecting duct result in the development of metabolic alkalosis.<sup>78</sup> Classically, hypocalciuria in Gitelman's syndrome was attributed to distal tubular Ca channel activation owing to the excessive transepithelial voltage polarization caused by defective NaCl reabsorption. However, recent studies in the Trpv5 knockout mice without epithelial Ca<sup>2+</sup> channels at the distal convoluted tubule concluded that hypocalciuria was secondary to the enhanced passive paracellular Ca<sup>2+</sup> reabsorption at the proximal tubule, after the electrochemical gradient caused by increased Na reabsorption.<sup>86</sup> In addition, mechanisms leading to hypomagnesemia are not well known.<sup>87</sup> Remarkably, patients receiving thiazides chronically show less severe hypomagnesemia than those with Gitelman's syndrome,<sup>75</sup> and, paradoxically, studies in isolated tubular cells showed thiazides stimulated Mg reabsorption per se.<sup>88</sup>

It has been proposed that hypomagnesemia occurs due to impaired Mg reabsorption through its specific channel secondary to the transepithelial voltage changes produced by defective Na reabsorption,<sup>89</sup> aldosterone hyperactivity<sup>90</sup> and associated hypokalemia.<sup>91</sup> Animal studies in the NCC knockout mice, an animal model of Gitelman's syndrome, have shown a downregulation of the epithelial Mg<sup>2+</sup> channel that may explain the pathogenesis of hypomagnesemia.<sup>86</sup>

### Differential Diagnosis

A differential diagnosis between Gitelman's and Bartter's syndromes is required (see earlier), mainly with Bartter type 3, which usually does not show manifest hypercalciuria.<sup>59</sup> It is an accepted rule that hypocalciuria is a characteristic finding of Gitelman's syndrome but in exceptional cases it also can be observed in the follow-up evaluation of patients with classic Bartter's syndrome.<sup>60,61</sup>

Autosomal-dominant familial hypomagnesemia, an inherited tubulopathy characterized by isolated hypomagnesemia with hypocalciuria, should be included in the differential diagno-

sis.<sup>82</sup> Laine et al<sup>92</sup> also reported on an apparent autosomal-dominant disorder that resembles Gitelman's syndrome. A father-daughter pair presented with hypokalemic metabolic alkalosis secondary to renal Cl<sup>-</sup> loss and severe hypomagnesemia caused by renal Mg<sup>2+</sup> loss, but these abnormalities were associated with marked hypercalciuria. Both patients developed dilating cardiomyopathy, which was fatal in the father and required heart transplantation in the daughter.

Sometimes it may be difficult to distinguish Gitelman syndrome from a Gitelman-like disease produced by hidden thiazides from a patient's self-administration. Measurement of drug levels in urine in highly suspected cases is indicated.<sup>93</sup> In addition, chronic salt-wasting tubulopathy caused by cisplatin nephrotoxicity also may resemble Gitelman's syndrome.<sup>94</sup>

### Treatment and Prognosis

Treatment relies on a K<sup>+</sup>- and Mg<sup>2+</sup>-rich food diet such as peanuts, nuts, chocolate, green vegetables, cereals, and oral K<sup>+</sup> and Mg<sup>2+</sup> supplements<sup>95,96</sup>; using parenteral supplementation only in severe episodes. Diuretics such as spironolactone, amiloride, and triamterene may represent an optional treatment, and prostaglandin inhibitors such as indomethacin in those refractory patients.<sup>97</sup>

Although traditionally considered a benign disease, there has been recent concern about sudden-death risk as a result of arrhythmia in affected individuals,<sup>98</sup> mainly in noncompliant youths.<sup>99</sup> Some clinicians have reported a reduced life quality in Gitelman's patients owing to chronic muscular weakness and less capability for sport training.<sup>78</sup>

### Inherited Tubulopathies With Hypokalemia and Hypertension

In general, inappropriate renal Na retention leads to volume-expanded hypertension.<sup>11</sup> Some hypertensive syndromes are inherited as single Mendelian traits and share a common feature: very low renin levels in plasma owing to inappropriate tubular Na<sup>+</sup> retention through the distal ENaC, and subsequent volume expansion.<sup>12</sup> These inherited disorders include Liddle's syndrome, glucocorticoid-remediable aldosteronism (GRA), and apparent mineralocorticoid excess (AME). Low-renin hypertension is confirmed if plasma renin activity (PRA) remains low after stimulation by salt depletion and/or furosemide administration.<sup>100</sup> Plasma aldosterone levels provide the guideline to establish the differential diagnosis among the following entities.

### Inherited Tubulopathies With Hypertension and Low Plasma Aldosterone Levels: Pseudohyperaldosteronism Liddle's Syndrome

Liddle's syndrome is a rare form of an autosomal-dominant disorder characterized early in life, and frequently by severe hypertension associated with hypokalemic metabolic alkalosis, low PRA, and suppressed aldosterone secretion.<sup>101</sup>

### Clinical and Biochemical Features

In their original report, Liddle et al<sup>102</sup> described a family syndrome of severe hypertension, hypokalemia, and metabolic alkalosis that mimicked hyperaldosteronism, but had exceptionally low rates of aldosterone secretion. Patients successfully responded to treatment, with amiloride and triamterene normalizing the blood pressure, reversing the renal K wasting, and correcting the hypokalemia as long as they maintained a salt-restricted diet. However, patients did not respond to spironolactone, a mineralocorticoid antagonist, indicating the presence of an intrinsic renal defect rather than a mineralocorticoid-induced disease. The renal anomaly was probed by the fact that the disease was caused by an intrinsic renal abnormality was demonstrated when the proband progressed to renal failure and the disorder normalized after receiving a cadaveric transplant.<sup>103</sup>

Affected children with Liddle's syndrome suffer polyuria, polydipsia, failure to thrive, hypokalemic metabolic alkalosis, and almost absent renin and aldosterone secretion.<sup>102</sup> Contemporary descriptions have shown that some patients may not have a clinical presentation of severe hypertension early in life, but retrospectively are found to have pseudoaldosteronism with long-standing hypokalemia and hypertension. Severe renal dysfunction is very unusual, but cardiovascular and cerebrovascular complications of hypertension are much more common within undiagnosed or untreated patients.<sup>104</sup>

### Genetics

A pattern of autosomal-dominant inheritance has been described, although some cases appear to be sporadic. Liddle's syndrome results from the constitutive activation of the renal ENaC as a result of mutations of the genes encoding the  $\beta$  subunit ( $\beta$  ENaC) or the  $\gamma$  subunit ( $\gamma$  ENaC).<sup>105,106</sup> Both genes (SNCC1B and SNCC1G) are located on 16p12. No abnormalities have been noted in the SNCC1A gene, located at 12p13, and encoding  $\alpha$  ENaC.

### Pathogenesis

Liddle's syndrome results from excessive renal tubular Na absorption through activation of the renal epithelial sodium channel. This channel is formed by 3 subunit proteins ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) having short cytosolic termini, 2 transmembrane domains, and a large extracellular loop. The  $\alpha$  subunit appears to be required for the assembly or function of the whole complex. The C terminal of each ENaC subunit contains a xPPxY motif that is necessary for interaction with the WW domains of the ubiquitin-protein ligase, Nedd4 to 2, thus triggering the internalization of the channel from the luminal membrane. The serum- and glucocorticoid-inducible kinase phosphorylates and inactivates the ubiquitin-protein ligase Nedd4 to 2.<sup>107</sup> Mutations of the xPPxY motif in  $\beta$  or  $\gamma$  subunits interrupt the interaction with Nedd4 to 2, reduce the endocytic retrieval of ENaC from the luminal membrane, and results in increased Na reabsorption, expanded plasma volume with resulting hypertension, and inhibited renin-aldosterone axis with secondary potassium wasting.<sup>108-110</sup>

It is possible that polymorphisms of the  $\beta$  and  $\gamma$  subunits of the ENaC are related to variations of blood pressure in

human beings<sup>111</sup> and may contribute to the high incidence of salt-related hypertension in African Americans.<sup>112</sup>

### Differential Diagnosis

At present, the finding of low renin hypertension in a child, especially with a provocative family history, leads the physician to consider other Mendelian forms of severe hypertension marked by early penetrance and substantial target organ damage, such as GRA and apparent mineralocorticoid excess, as discussed later in this article.<sup>100</sup>

### Treatment and Prognosis

K-sparing diuretics are the drug of choice for patients with Liddle's syndrome, although spironolactone is not useful. Some pedigrees are more responsive to amiloride and some are more responsive to triamterene.<sup>104</sup> In all cases, the use of amiloride and triamterene are enhanced by dietary salt restriction, reflecting the known competition of Na and those diuretics at the level of the ENaC channel.<sup>113</sup> Some patients need to associate vasodilators and/or  $\beta$ -blockers with the treatment to achieve normal blood pressure. Thiazides hypothetically could help with the Na<sup>+</sup> retention, but they usually worsen the renal K wasting.<sup>104</sup>

### AME

AME was first defined as a syndrome in 1977. New et al<sup>114</sup> reported a 3-year-old girl with hypertension, hypokalemia, metabolic alkalosis, and suppressed renin but with low serum aldosterone and decreased secretion of all known sodium-retaining corticosteroids. Later Monder et al<sup>115</sup> described children with this form of severe and early hypertension who responded to spironolactone or to a low-sodium diet, suggesting the disease was mediated by the mineralocorticoid (type I) receptor.

### Clinical and Biochemical Features

An affected child's clinical picture is characterized by low birth weight, polyuria and polydipsia, failure to thrive, severe hypertension, and hypokalemia. Hypertension is difficult to treat and shows a high degree of morbidity/mortality, with early target organ damage.<sup>116</sup> Biochemical profiles show metabolic alkalosis and severe hypokalemia. PRA is low, suggesting a volume-expanded type of hypertension, with response to salt restriction.<sup>117</sup>

Case reports of individuals with AME and a less-severe phenotype with mild hypertension also have been published.<sup>118</sup> Renal cysts, nephrocalcinosis, rickets, and hyperparathyroidism are reported sporadically.<sup>119</sup>

### Genetics

AME is caused by autosomal-recessive mutations in the HSD11B2 gene situated on chromosome 16q12, and encoding the 11- $\beta$ -hydroxysteroid dehydrogenase type 2 (11- $\beta$ -HSD2) activity, an enzyme that protects human beings from cortisol intoxication.<sup>117,120</sup> Most patients are homozygotes for one of the different mutations; few compound heterozygotes have been identified.

### Pathogenesis

This disease is explained by the fact that the selectivity of mineralocorticoid action is determined not by the mineralocorticoid receptor (MR) ligand-binding site, which is nonspecific and does not distinguish between aldosterone and cortisol,<sup>121</sup> but by the action of an intracellular enzyme, 11- $\beta$ -HSD2, that systematically inactivates glucocorticoids and thereby protects the MR from saturation. This enzyme is active in the collecting tubular cell, and catalyzes the conversion of cortisol to cortisone, which has much less affinity for the MR. In fact, the 11- $\beta$ -HSD2 enzyme confers specificity on the MR by converting biologically active glucocorticoids to inactive metabolites. Mutated genes in AME produce an excess of cortisol that saturates the MR, causing a hypermineralocorticoid state that results in Na retention, subsequent volume expansion, and secondary plasma renin and aldosterone level suppression. In addition, prolonged availability of cortisol results in adrenocorticotrophic hormone (ACTH) suppression and, therefore, decreased adrenal cortical secretion.<sup>122</sup>

### Differential Diagnosis

Typical patients with AME evidence an abnormal ratio of cortisol to cortisone metabolites (tetrahydrocortisol plus aldotetrahydrocortisol/tetrahydrocortisone), and by an exceedingly diminished ability to convert cortisol to cortisone (only 0%-6% versus the normal rate of 90%-95%).<sup>117,123</sup> In children and adults hypertension may be produced by congenital adrenal hyperplasia owing to 11 $\beta$ -hydroxylase deficiency, an autosomal-recessive entity, caused by mutations of the CYP11B1 gene. Accumulation of corticosterone and cortisol precursors with net mineralocorticoid effect, lead to renal sodium retention, volume expansion, and subsequent suppression of RAAS with low renin and aldosterone levels.<sup>122</sup>

An acquired syndrome of pseudohyperaldosteronism, in all ways identical to 11- $\beta$ -HSD2 deficiency, has been described after licorice ingestion.<sup>124</sup> Natural licorice, an extract of *Glycyrrhiza glabra* root, contains glycyrrhizic acid, which is a potent inhibitor of 11- $\beta$ -HSD2. This complication is observed very infrequently in children.<sup>125</sup> Other causes of secondary pseudohyperaldosteronism to be excluded are produced by drugs. Carbenoxolone, an antiulcer drug, inhibits 11- $\beta$ -HSD2 competitively, leading to Na retention as well. The possibility of an AME syndrome caused by extensive use of creams or nasal inhalers containing 9- $\alpha$ -fluorinated corticoids also should be ruled out.<sup>2</sup>

### Treatment and Prognosis

Spironolactone, an MR blocker, is the drug of choice. After its administration, blood pressure normalizes, urinary Na levels increase, and PRA becomes detectable as volume balance is restored with the treatment.<sup>123</sup> In addition, thiazides may help to control hypercalciuria and hypertension with a lower spironolactone dose. The defect disappeared in the very few patients who needed a kidney transplantation.<sup>126</sup>

### Familial Hypertension Secondary to Activated Mineralocorticoid Receptor

Geller et al<sup>127</sup> reported on a family whose proband, a 15-year-old boy, had severe hypertension and suppressed plasma aldosterone levels. This patient and 11 of 21 at-risk relatives, all hypertensive, were heterozygous for a missense mutation substituting leucine for highly conserved serine at codon 810 of the mineralocorticoid receptor. This mutation resulted in a gain-of-function of the receptor and alteration of its specificity because it also was activated by progesterone. Carriers not only presented severe early onset hypertension but, although normokalemic, tended to have lower plasma K values and manifested severe hypertension during episodes of pregnancy.

### Inherited Tubulopathies With Hypertension and Normal Plasma Aldosterone Levels

#### GRA

GRA, or glucocorticoid-remediable hypertension, also named *familial hyperaldosteronism type I*, is an autosomal-dominant disorder that is characterized by hypertension via the mineralocorticoid receptor.<sup>128</sup> Characteristically, aldosterone secretion is solely under the control of ACTH. It appears to be the most common monogenic form of human hypertension, although it frequently is underdiagnosed.<sup>129</sup>

#### Clinical and Biochemical Features

This entity was first described in a father and son with hypertension, low PRA, and increased aldosterone secretion responsive to dexamethasone.<sup>130</sup> Clinical features include early onset hypertension, usually in adolescence, although studies in large families have shown extraordinary phenotypic variability. Some affected individuals may even remain normotensive until late in life.<sup>131</sup> The hypertension varies in severity but typically is refractory to standard antihypertensive drugs. Laboratory findings include normal to low-normal serum K levels, but hypokalemia may be attributed to thiazides.

#### Genetics

This autosomal-dominant disease arises because of a chimeric gene that is formed at meiosis after the genetic recombination between 2 homologue adjacent genes on chromosome 8q22, CYP11B1, and CYP11B2, encoding the enzymes 11- $\beta$ -hydroxylase and aldosterone synthase involved, respectively, in the final pathways of cortisol and aldosterone synthesis within the adrenal cortex. The resulting chimeric gene duplication fuses the regulatory elements of 11- $\beta$ -hydroxylase, and the coding sequence of aldosterone synthase.<sup>132</sup> As a result, aldosterone is synthesized ectopically in the adrenal zone fasciculata under ACTH control, rather than angiotensin II, with resultant hyperaldosteronism and suppression of angiotensin-II-stimulated aldosterone production in the zone glomerulosa.<sup>133</sup> In addition, the chimeric gene product converts cortisol to its 18-oxo and 18-hydroxy metabolites, causing a pathognomic urinary steroid profile.<sup>100</sup>



## Diagnosis

The traditional screening test for GRA diagnosis is the dexamethasone suppression test. A significant reduction in plasma aldosterone after at least two days of treatment with dexamethasone is characteristic of this form of aldosteronism.<sup>134</sup> The specific diagnosis of GRA can be confirmed by increased 24-hour urinary levels of steroid metabolites, 18 oxotetrahydrocortisol, and 18 hydrocortisol. A high ratio of 18 oxotetrahydrocortisol/tetrahydroaldosterone in the urine is pathognomonic for this entity.<sup>100</sup>

## Treatment and Prognosis

Glucocorticoid monotherapy is the drug of choice to suppress the activity of the chimeric gene suppression.<sup>129</sup> However, older children usually require more intensive treatment and a wide variety of nondirected antihypertensive agents, such as angiotensin-converting enzyme inhibitors, calcium channel blockers,  $\beta$ -blockers, and diuretics, often in combination are needed to achieve blood pressure control.<sup>135</sup>

## Inherited and Acquired Disorders With Hypertension and High Plasma Aldosterone Levels: Hyperaldosteronism

The syndrome of primary aldosteronism is characterized by autonomous or angiotensin-independent aldosterone excess resulting in hypertension and suppressed renin activity with or without hypokalemia. The ratio between aldosterone/renin levels in plasma constitutes the screening test of this disorder. It comprises the aldosterone-producing adenoma, and bilateral adrenal hyperplasia or idiopathic hyperaldosteronism.<sup>136</sup>

In summary, an increasing number of distinct inherited tubulopathies with impaired sodium and volume balance result in hypokalemia and metabolic acidosis. Differential diagnosis may be established according to the RAAS and blood pressure status, although definitive diagnosis requires specific molecular genetics. Despite its rarity, better understanding of the pathogenesis of these entities may help to identify protective or risk factors for hypertension in the general population.

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